Hello, my name is Gary Cutting, and I'm going to speak on the topic of human Mendelian genetics. Mendel, and his study of peas, have had implications for genetics and, particularly, for modern medical genetics that are profound. Indeed, much of what Mendel learned from peas has enabled us in the past decade or so to find genes that are responsible for a variety of genetic disorders, primarily those caused by defects in an individual gene. And so, a thorough understanding of Mendel and his contribution to genetics and how those contributions have now been applied to human genetics is essential, and also a thorough understanding of how geneticists utilize such tools as the pedigree and pedigree analysis is also essential to an understanding of how genetics can help the study of any diseases.

So, who was Gregor Mendel? Well, he was an Augustinian monk who lived in Bruno in what is now the Czech Republic, and he was very interested in horticulture and was under the tutelage of a senior monk who was a scientist and who taught Gregor the importance of hypothesis-driven science. And Gregor was very interested in the traits that peas carried and how they could be passed from one generation to another. And in this paragraph, taken from a letter to Carl Nageli, who was actually a disbeliever and who Gregor was trying to convince, he pointed out the following important fact:

In 1859, I obtained a very fertile descendant with large tasty seeds from a first-generation hybrid. Since, in the following year, it's progeny retained the desirable characteristics and were uniform, the variety was cultivated in our vegetable garden, and many plants were raised every year up to 1865.

Now this is something that many people who raise plants, or who breed dogs, understand — that traits can be passed from one generation to another, and it is actually possible to breed plants and animals specifically for those traits.

Well, the same things apply to humans and, indeed, what Mendel discovered in peas, we're going to see, can be applied to humans, and humans with diseases that segregate in families, and the rules that he discovered by which those traits were passed, apply as much to peas as they do to humans.

In his seminal paper, which was lay hidden actually for fifty years until it was rediscovered by a series of mathematical geneticists, he pointed out three very important points. First, the characters or traits from parents pass as unmodified units, which we now call genes, to successive generations according to set ratios. And this was a profound insight because, up to this point, the concept that one could alter the traits you passed on by the things you did during your lifetime, called the Lamarckism Hypothesis, was reigning supreme. The concept that what you did in life did not affect what you passed on to successive generations was truly new and Mendel, from his work, deduced this concept, which we now know to be, of course, completely correct and formed the foundation of all genetics.

Second, individuals passed two factors, and those factors we now call alleles, one of which is received from each parent. And it makes no difference whether any one allele is

inherited from male or female; they both contribute in the same way. So, the concept that these genes and variations in these genes called alleles are unitary, invariant and passed consistently from generation to generation was the major contribution. And because they followed these rules, mathematics could be applied.

And third, alleles are sometimes expressed and sometimes concealed, but never lost. So, the concept that these genes and what are passed from generation to generation are consistent and unvarying, but may or may not exhibit their features in the individual character was an essential insight of Mendel's.

Based on these insights, two laws have been put forth, which we call Mendel's Laws, and they illustrate the principles of Mendelian genetics. First is the law of segregation. It is the separation of two members of each pair of alleles possessed by a diploid organism — that's an organism that would have two sets of alleles for every gene that occurs in their body — and these alleles are passed into different gametes and into different offspring.

Second is the law of independent assortment, and that is that the members of different pairs of alleles are assorted independently during gametogenesis, and the subsequent pairing of male and female gametes is at random.

I will explain these two concepts in the next few slides using Punnett squares. Indeed, the Punnett square is one of the tools that geneticists like to use in illustrating these two laws, and it's also the time during, while you're a student, whether you decide to continue in genetics or not, because these are the simplest forms of Punnett squares but, as you could imagine, they can get quite complex depending on how many traits you're going to be tracking.

So, first we'll talk about that first law — segregation — and alleles segregate and, I will tell you later, genes assort. So, first we'll start with the alleles segregate. So, here we have two individuals, male and female, who are hybrids. They are each are carrying a capital A and small a, which are two versions, two alleles of a particular gene, capital A gene and the small a gene. Into their gametes, due to segregation, they will pass either capital A or small a. Then when the gametes combine, you will have several, you'll have actually three combinations, homozygous for capital A, homozygous for small a, or the heterozygous state.

Why is this important? Well, if capital A contributes to the presence of the particular trait, it could be a disease, it could be a particular feature, it could be hair color, eye color, height, or something like that. Then capital A will dictate whether or not the feature is present. So, in this simple experiment here, on average, three out of four individuals that might be born to these hybrid individuals, couple, will have the trait — the individual who has two copies of A and the two individuals that are heterozygous that have one copy of A — and so the ratio will be three affected to one unaffected.

And this is where mathematics applies because you've got these units that are consistent and passed in a consistent way so that certain rules and laws can be applied, and so

mathematics can also be applied. So mathematics is actually a very important part, the way in which genetics is treated. And, of course, as one can see, this would be the ideal situation where there were four individuals observed so you could see that three out of four would be affected. Of course, in usual situations in medicine, not all families will have four children, for example, if they're segregating a dominant disorder in order for you to identify that a particular trait might be dominantly inherited. However, by looking at a series of families with the same phenotype, you can start deducing this type of information.

Now, recessive inheritance is a situation in which the trait is only expressed in the individual expressing, or having, only small a, so they would have to have both copies being small a and not having the large A which will contribute to a normal phenotype. And so, in this case, again you can work out straightforward mathematical equations as just one affected to three unaffected.

And finally, an X-link recessive inheritance — this is where the trait of interest would be on the X chromosome. A male would only have one X chromosome and a Y as I've indicated on the top, so that male would be AY, and he would be normal, and the female would be A, small a, she's heterozygous and, as you can see, one out of four, on average, of their offspring will be affected, and that affected will be a male, as shown in the red box. So, the females will be unaffected. In fact, one out of the two females will be a carrier of that small a, and similar to her mother, and the males will have a ratio of one affected to one unaffected.

So, that's alleles segregating, and now genes assorting, and this is a little bit more straightforward because we're just looking at genes going into different gametes. And so, here's an individual who's heterozygous in two genes — the A gene and the B gene — and this individual carries a large A, small a, big B, small b, and into his gametes, the genes will assort. And they sort independently so that his gametes can have either large A, large B, large A, small b, small a, large B, or small a, small b, as you can see. And so there is no bias of the gametes acquiring a particular allele because the genes are independently assorting.

There is one caveat that should be obvious to most in the audience that, obviously, genes located close together, on the same chromosome for example, would not assort, because they're going to go together if they're on the same chromosome. In most cases, the genes are on separate chromosomes, and that's why they assort independently. Of course, Mendel had no idea about the concept of chromosomes or even any clue as to where their genetic information was stored, and so this all had to be derived from his calculations of the traits he saw in peas. But, indeed, when the chromosomes were discovered, a mechanism for gene assortment then became immediately apparent.

So, the primary tool of geneticists is the pedigree and, in order to gather a pedigree that's going to be useful to determine whether a Mendelian disorder is being transmitted in the family, one needs several pieces of information.

First of all, you need at least three generations. You absolutely must have accurate and complete phenotyping of all the members of the family. Gain the history on, say grandma, from the patient is of some use, but it's much more useful to actually acquire the chart and look through the chart of that individual after, of course, proper consenting and so forth, to make sure that if the patient said this individual was affected, or just as importantly not affected with a particular trait, you can verify it.

The age of relatives is also important because diseases sometimes have different ages of onset, and so you need to know how old the individuals are in the pedigree so that you could determine whether or not some individuals may actually be too young to actually see the onset of a particular disorder. You must have the correct relationships, and that does mean sometimes asking some tough questions. One example is consanguinity, the situation in which two individuals who are related form a union and have children is very important to identify. In addition, so is non-paternity, and that's sometimes a more difficult question to ask but, under the appropriate circumstances, can be asked, and should be asked, so that one can ascertain that the presumed father, or the indicated father is, indeed, the individual who is contributing genes to the pedigree.

And, finally, ethnic and racial history are important because certain disorders, as we'll learn in a few slides, certain disorders tend to be more common in certain groups.

What are the elements of a pedigree? We show here the symbols that should be familiar to most members of the audience. The squares indicate the males, the circles the females; a colored circle or square will indicate an affected individual; a diagonal line across any one of the symbols indicates a deceased individual. Two lines between two individuals, as shown by the male and female on the second row, indicates consanguinity. Single lines just indicate relatedness.

On the third line we see two diagonal lines joining each other, two males, and that's a symbol for dizygous twins, and next to them is a small diamond, and that small diamond indicates a miscarriage. There are a variety of additional symbols that the geneticist will use, but this illustrates some of the more common ones.

In order to identify individuals in different locations in the pedigree, we denote generations by Roman numerals, and individuals within that generation by Arabic numerals.

So, here is a pedigree from a family in which we have indicated in orange affected individuals, and you can see that we have, in the first generation, an affected male who has approximately half of his children affected with the same disorder, and then one of his affected children, the oldest daughter, has one of her daughters affected with that condition. And this pedigree illustrates most of the features that one would see with autosomal dominant inheritance.

The trait appears in every generation without skipping. Second, the offspring of an affected person has about a 50 percent risk of inheriting the trait. So, one can see that the affected male in the first generation has passed his trait on to about half of his offspring. So, each of the offspring in each occasion had a 50 percent chance of acquiring that trait.

Unaffected family members do not transmit the trait. The key issue behind autosomal dominant inheritance is that, if you have the trait, you have the abnormal gene. If you don't have the trait, you don't have the abnormal gene. Therefore, you can't pass it on. So, for example, if you look at generation two, that would be the second line, and look at the third male, you will see that he is not affected with the trait and neither are his two children. Of course, you could argue, well there's only a 50 percent chance with each child not being affected with this trait and having two individuals not affected with that trait is a chance of about 25 percent, how would one be sure. Well, obviously with larger size pedigrees, you could be more sure that the trait is not being passed on in some other manner of inheritance.

So, there lies one of the keys behind pedigree analysis, there's only so much you can deduce from a certain number of individuals. The larger the pedigree, the more individuals who have been properly and fully phenotyped, and the more generations you have studied, the more sure you can be of the inheritance pattern. There are a number of pedigrees, though, in which one has to be careful because using a small size pedigree entails a risk of over-interpreting the inheritance pattern.

Finally, as you can see as illustrated by the pedigree, the occurrence and transmission of the trait is unaffected by sex. So, males and females can pass the trait equally.

Some characteristics of autosomal dominant inheritance. Well, first there is a high rate of new mutations in disorders that affect reproductive fitness. This is generally due, but not always due, to the fact that the mutations arise in the male during spermatogenesis, so these are generally point mutations, or small alterations in genes, that give rise to particular disorders, and they have given the example here of achondroplasia. Eighty percent of cases of achondroplasia, this dominant disorder, are due to new mutations.

Codons. As you can imagine, if you have one dominant gene, you could have one dominant allele, I should say. You could have another dominant allele, and then the two dominant alleles could work together, and a good example of that is the A and B blood groups. The O allele of this blood group is recessive to both A and B, so you can be an AB blood type, you can be an AO, but the A being dominant to the O, or you can be BO, B being dominant to the O, but A and B being co-dominant.

Okay. Also, recessive inheritance is the other side of the coin from the dominant inheritance. And we see here a family in which individuals in one sibship are affected and only in one generation and, indeed, this is the hallmark of a recessive inheritance. The trait appears only in sibs, not in parents or other relatives. The recurrence risk for the same set of parents is 25 percent, or one in four, and that's based on the Punnett square that we showed earlier

because, again, the same way traits are transmitted, recessive traits are transmitted in peas, recessive traits in humans are transmitted the same way.

Either sex can be affected and, finally there is, in recessive disorders, not uncommonly consanguinity. In other words, a relationship between the parents that increases the chance that rare recessive alleles will come together again in offspring. So, as you can see here in the family with the two affected children, the father and the mother are related to each other and, due to the fact that they're related to each other, they could inherit a recessive gene, which was not exhibited in them, because it's recessive, but we show the pattern of inheritance of recessive gene by that black dot. So the black dot shows you that it started with a grandfather, was transmitted down to two of his children, and then finally to the mother and father of the two affected children. So, consanguinity is a key feature in recessive disorders.

Now, one thing that's important to emphasize, because this question comes up a number of times, why are certain traits, particularly recessive traits, not lost in the population if that particular trait or disorder is lethal? New mutations do not occur that often although some occur in recessive disorders as they do in autosomal dominant disorders. So, certainly, we're not maintaining the recessive genes by new mutations, but we are maintaining them by the heterozygotes, the carriers. And, in some cases, the carriers can have an advantage over those who are not carriers, and the perfect example of this is sickle cell disease and resistance to malaria.

And so, if you increase the frequency of individuals who are heterozygote carriers in the population, then you will increase the frequency of those affected with the disorder, those who are homozygous for the recessive allele, but you will never get rid of that disorder because the heterozygotes will always maintain the gene in the recessive state because they are phenotypically normal. And, unless they have a disadvantage, a very strong disadvantage, then it is virtually impossible to rid the population of recessive alleles.

And, indeed, recessive alleles in certain circumstances, as I pointed out, like sickle cell disease, may have been advantageous to humans at some point during our evolution. This is actually a very good way for us in genetics to explain to sometimes devastated families the role of carrying certain genes in the population that they may actually be some advantage to these genes, and this is why they showed, and unfortunately have now been affected by the disorder, but that these genes have been around for millennia and that it is impossible for us to eliminate these genes from the population and that the genes probably had some advantage at some point.

Another issue related to autosomal recessive disorders is the frequency of recessive alleles in a stable population, and this is a population where there isn't a lot of flux of new people coming in or going out from the population, so in a stable population, you can estimate the frequency of recessive alleles using this formula called Hardy Weinberg. And this is very useful because if you have a particular incidence of a disease or recessive disease, for example, and let's say it's one in 2,500, if you take the square root of one in 2,500, that's one in fifty,

you now have the gene frequency in that population. And it's derived from, as you can see, the p square plus 2 pq plus q squared, q squared being the frequency of individuals with the recessive homozygous recessive genotype. And this is a common tool that we use in recessive disorders that calculate the frequency of a gene in the population and the frequency of carriers because carriers are calculated from the 2 pq portion of that formula and, of course, p squared being the homozygous for the wild-type allele.

Finally, and the reason why racial and ethnic-type information is important is that there is a high frequency of rare recessives in certain genetic islets. One example is Tay-Sachs disease in Ashkenazi Jews; another would be tyrosinemia in French Canadians. There are many, many examples of recessive alleles that were introduced into somewhat isolated populations by founders, and that's called a founder effect, and due to a certain degree of continued mating amongst that group, an exclusion of individuals coming into that group, particular alleles became higher frequency in that select population than in the general population. So, studying certain disorders that are seen at relatively high frequency, but only in certain populations, has been a very successful way of identifying the molecular basis of certain disorders.

Finally, allelic heterogeneity, and this is an interesting phenomena and not unexpected from a basic science point of view, and that is that different combinations of alleles give rise to variant presentations of the trait. Here we show a graph pointing out different combinations of alleles on the bottom. You can see there's a mutation R261Q; that's just a change in one amino acid. Null, that's a mutation that knocks out the function of this protein, and the protein that we're looking at in this case is phenylalanine hydroxylase which, when defective, gives rise to PKU, or phenylketonuria. And you can see that they have individuals that carry either the R261Q mutation and null, or homozygous for R261Q, or carry some other mutations such as Y414C. What's interesting is that you can see in orange are the individuals who have PKU with those different combinations of alleles, but there are also individuals who have variant PKU, like a milder form of PKU and, finally, those who don't have PKU; they just have an elevation in phenylalanine, which is called hyperphenylalanemia.

And you can see that, with a certain combination, like on the left, R261Q with null, most of the individuals, but not all, have PKU. Then if we move to the next genotype, you can see more individuals now have variant PKU, but very few have hyper-phe. Finally, with the next combination, almost all of them have variant PKU. And in the last combination, we now see that none of them have PKU, and they either manifest variant PKU or hyper-phe. They all have defects in the phenylalanine hydroxylase, yet they're giving rise to what are different phenotypes. And this is a phenomenon we see in all Mendelian disorders, but is particularly a phenomenon associated with recessive disorders because of the combinations that you can get and the variety of combinations you can get that can create these different phenotypes.

Okay. So we talked about autosomal dominant and autosomal recessive inheritance. Now, we'll move on to sex-linked inheritance forms, and we'll start out with X-linked recessive. Here's a pedigree in which we have only males affected, and we show by the black

dot that we have females that are shown to be carriers. The principles of X-linked recessive inheritance are as follows:

First, the instance of the trait is much higher in males. Second that the affected male transmits to all daughters and, on average, to half of his grandsons. So, if we take a look in this generation, or this pedigree, at the first generation, we have an affected male passed on to actually, in this case, as you can see, both of his daughters, because it's on his X chromosome, and he passes, of course, only his X chromosome to his daughters and only his Y chromosome to his sons, so both of his daughters are carriers. And, of those, the woman, the second daughter, I apologize, second daughter has passed a trait to one of her sons and one of her daughters and then that daughter has passed that trait to her son. The males carrying the trait are the ones that are affected. And if you had a large enough pedigree, you would see that that grandfather, indeed, would have approximately half of his grandsons affected with the same conditions that he manifests.

There is no male to male transmission in a single generation, and we can emphasize the point that the trait is on the X chromosome and males pass the Y chromosome to their sons, so they cannot pass an X-linked trait to their sons.

And affected males are usually born to unaffected parents, and the mother may have affected male relatives. You have another feature of X-linked recessive inheritance, and you can see that that's shown or illustrated in this pedigree.

What are some characteristics of the X-linked recessive disorders? Well, female carriers show variable expression of the trait, and this is something that we recognize is generally due to skewed X inactivation, so if you reach back to your days in biology, you'll remember that, in females, with two X's, one of the X's is inactivated in every cell of the female, and the inactivation is random, and if it just so happens that that individual, that woman, carries a trait, an X-linked trait, on one of her X chromosomes and has the normal trait, or normal gene, on the other X chromosome, then depending on how she inactivates her X chromosomes would depend whether she manifests that particular trait. If that gene is normally expressed in the liver, for example, in factor 8 hemophilia, or hemophilia A, which is an excellent trait, and the factor 8 is expressed in the liver, and in a woman carrying that trait happens to have a skewed inactivation of the X chromosomes bearing the normal gene, the normal factor 8 gene, then her cells will primarily have X chromosomes bearing a mutated factor 8 gene. Therefore, she will not produce normal factor 8, and it will manifest with a mild hemophilia. And this is a characteristic we see of X-linked disorders. Hemophilia A is one example; adrenoleukodystrophy is another; Duchenne Muscular Dystrophy a third.

One-third of lethal traits, X-linked traits, are due to new mutations, follows predictions from Haldane, a mathematical geneticist, and this is a well-recognized phenomenon that, in situations such as hemophilia, we know that one-third of those boys with that trait have it due to a new mutation that occurred, usually in the grandfather, was then passed on to their carrier unaffected, or maybe mildly affected due to skewed inactivation, mother, who

then passed it to them, but it was a new mutation trait that can't be traced further back in the family.

And finally, a high proportion of disease-associated mutations, are genomic re-arrangements deletions, insertions, and so forth. Usually deletions and insertions are not seen in recessive disorders but, in the case of the X chromosome where the male has no opposing X chromosome with a second gene to counter a genomic re-arrangement that may occur in the X chromosome, then the individual would exhibit the trait. And so, again, I'll give you two examples — Duchenne Muscular Dystrophy and hemophilia A. A large proportion of those cases are due to genomic re-arrangements.

Now, you can have recessive X-linked inheritance, but you can also have dominant inheritance. This is much more rare and usually due to the fact that the dominant inheritance is usually lethal to the male, but there are some disorders, hypophosphatemic rickets is an example, and, here in this pedigree, you see both males and females affected, and it exhibits a number of the traits of this form of inheritance. One is that affected females are more common than affected males, and you can see here there are five affected females and two affected males, and that's characteristic of this type of inheritance pattern.

Females often have milder and more variable features. And, again, that's due to X inactivation issues. Affected heterozygous females transmit to half of offspring regardless of sex, and affected males have affected daughters but no affected sons. Again, because males will pass the Y chromosome to sons and can only pass the X chromosome to daughtersbut, of course, because it's X that's dominant, all of their daughters, all the daughters of an affected male will be affected themselves.

Now, another example which can sometimes, and we call it a sex-linked trait because it is, on the sex chromosomes X and Y, but actually can masquerade as autosomal recessive inheritance, and that's because our sex chromosomes retain regions that are similar between the X and Y, and they're called a pseudo autosomal regions and, as you can see, they occur in the tips of the X and the tips of the Y. And these regions are frequently interchanged during meiosis such that genes in these regions can cross over from the X to the Y. And so, if you're doing a pedigree, it really can make it difficult to sort out whether you have male transmission or transmission that is going along with the X, and so usually one concludes that it's not X-linked but that it is autosomal and likely autosomal recessive.

So, here's an example, of Prader-Willi Syndrome, and males inherit from males. That's because the gene response for this disorder is in the pseudo autosomal region, and so it's on the Y chromosome and it, therefore, it rules out X-linked inheritance, and males can inherit from females. So that shows it must be on the X chromosome, so how can it be on the X and the Y. Well, let's say to the gene responsible for this disorder called SHOX. SHOX is in the pseudo autosomal region and mutations in SHOX order stature that gives rise to this syndrome, and then SHOX with a mutation can be exchanged between the X and the Y with any pedigree. Therefore, you see the pattern of inheritance I just indicated.

So, Mendel's rules have not been violated and, in fact, we'll see from a number of other examples we'll go through that unitary inheritance of these traits is not violated. It's just there are some exceptions based on the structure of our chromosomes or the way in which genes are expressed.

So, what are some of the other complications in the interpretation of Mendelian traits? And this is especially important to consider in human genetics because rarely do we have the opportunity to have large pedigrees where we have many affected members and many unaffected members and multiple generations, and sometimes we do, but not always. We certainly can't do selected breeding as Mendel did with his peas. People tend to frown on those kind of things. So, geneticists have to be, take advantage of information that's available to them.

And, finally, there are now opportunities with the advent of molecular genetics to now test and dissect out the proposed patterns of inheritance and, in fact, sometimes we discovered, through the use of molecular genetics, that some forms of Mendelian inheritance actually were different than initially proposed.

So, first we'll talk about penetrance and expressivity, and then we'll move on to pleiotropy, parent of origin effects, genetic heterogeneity, then non-allelic interaction, anticipation, mosaicism and modifier genes, and I can just say this is not a complete list. There are several other complications that can occur. I've just summarized the most important ones.

So, let's talk about penetrance first as expressivity. So penetrance is the fraction of individuals with a genotype known to cause disease who actually have the disease, whereas expressivity is the extent to which a genetic defect is expressed. The trait may vary from mild to severe, but never completely unexpressed in those with the genotype. And the concepts of penetrance and expressivity applies to all Mendelian forms of inheritance, but more so to autosomal dominant inheritance than the other forms. So, you can kind of think of penetrance and expressivity in the following terms; that penetrance is kind of like a light switch with on and off or none whereas expressivity is perhaps a dimmer switch, a difference in severity or gradation.

So, here's an example of penetrance, a trait that's being inherited through this family, the gene, the abnormal gene, or abnormal allele, is shown in orange and you can see that it's a dominant condition. We have members of each generation affected. There is a male to female and female to male transmission, and you can see that, out of eight individuals that carry the mutation, there are actually only six that manifest disease, and those that manifest disease are the ones shown with a black dot. And so, in this case, we have 75 percent penetrance, and we call this genetic trait, this autosomal dominant disorder, as incompletely penetrant.

Now, as an example of expressivity, this is a family transmitting Marfan syndrome, and we have in the red symbols individuals who carry a mutation and, in each case, they also

manifest disease, so they have the mutation and Marfan syndrome, but the clinical features vary considerably. You can see the grandfather of this pedigree has had aortic root dissection, he has myopia, he is tall, has a high palate, and has a score of 9 and 9 on the Brighton scale, which evaluates the integrity of his connected tissue.

So, he has full-blown Marfan syndrome but, if you look at other individuals in the family, we have one of his sons who is actually shorter, 5 foot nine. I actually think that's a pretty good height myself, a dilated aorta, but no other manifestation; a daughter who is actually taller than this boy (this is the oldest daughter in this family), and she's 5 foot ten, and the only manifestation she has is dislocation of the lens. And you see that two other individuals have various features. So, this is variable expressivity. One individual with full-blown features of the condition; others with partial features of the condition, and so this illustrates very nicely variable expression.

Okay, pleiotropy. So one gene more than one effect, and this is something that we appreciate for a variety of disorders. I would say that this applies more often to autosomal recessive conditions, though certainly can be seen in dominant X-linked, but this is something we think of more if we go to autosomal recessive.

So, here we have the CFTR gene, and when that gene is defective, it gives rise to a relatively common autosomal recessive disorder, cystic fibrosis, and we know that you can have classic and non-classic forms of that disease. Individuals with non-classic forms of the disease have some manifestations of the feature, but not all manifestations of the disorder that you see in the classic form of the disorder.

But defects in the CFTR gene can also give rise to a separate condition of male infertility, called congenital bilateral absence of the vas deferens, or CBAVD. It also can give rise to isolated sweat gland dysfunction or idiopathic pancreatitis and, finally, chronic rhinosinusitis. So this is the way in which one gene, and defects in that gene which cause abnormalities of the function of the CFTR protein can be manifest in different tissues and manifest as different phenotypes. And the reason we understand that this is a curve is that if you look at the amount of function that these mutations leave in the protein, you can see a pretty clear association with the phenotypes. Those have very little residual CFTR function, have cystic fibrosis. Those that have higher levels of function have the male infertility phenotype. Those that have a step higher of function developed pancreatitis. And, again, all these features — pancreatitis and CBAVD — could be seen in CF, but they also can be seen in isolation as shown here. Finally, isolated sweat chloride is associated with high function and, finally, patients with a relatively common disorder in the population, sinusitis, can have disease due to dysfunction in this gene. And, of course, going above 50 percent in function — that's where you are at a heterozygote state — you have a normal phenotype.

This pattern we're seeing here, which has been worked out over the past decade for cystic fibrosis, I think is going to be the rule rather than the exception for most Mendelian disorders.

Now let's switch and go to parent of origin effects, so it's important as to which parent you inherit your abnormal gene in certain disorders. So this is the phenomenon of different expression allele depending on parent of origin, and a good example is chromosome 15 and, in particular, in a region of Q11 to Q13, and that region is involved in two different phenotypes. The phenotype that you develop is dependent on which parent you inherit the abnormal gene from, and the process, the molecular mechanism, that underlies this phenomenon, is imprinting. Imprinting is a process that normally occurs in gametes so I'll illustrate this.

So, regardless of the mechanism that creates the mutation — whether it's a deletion, whether it's we'll call it uniparental disomy, whether it's point mutation, whether it's imprinting center mutation, four different mechanisms showing here — it really doesn't matter. What matters is whether you get the abnormality from dad or the abnormality from mom. And on the left side here of this slide, on the right side of this slide, you'll see that we have a male and female, and they're shown here with two different chromosome 15's in pink and blue, and there's a certain amount of imprinting, this is methylation that occurs on the chromosome that once you go through gametogenesis, it's erased, and then both of those chromosomes are re-imprinted as coming from dad. And the same thing happens in the female. Now gametogenesis, it's re-imprinted as coming from mom, hence the use here of blue and pink.

So, now we can see that the blue gene, or blue chromosome, in the sperm is now contributing to an individual, and that individual, if they have an abnormality on chromosome 15, will develop Prader-Willi Syndrome. So, if the paternal contribution, I'd say gene or a series of genes is missing, you develop Prader-Willi. You take the exact same region but now the missing material is actually coming from mom, you develop Angelman. And that is due to the fact that there are imprinting differences between the male and female chromosomes in this region of chromosome 15 and you end up developing quite different phenotypes. There's Prader-Willi, for example, this boy you see he's quite heavy, he had feeding problems at infancy, he became rather obese, he has small hands and feet, hypogonadism, and has mental retardation. And yet, missing the same region but coming from mom, you develop Angelman and they exhibit early speech delay, distinctive facial appearance, mental retardation, seizures, and laughter outbursts – a different phenotype altogether than what we see in Prader-Willi.

Genetic Heterogeneity. If things weren't fun enough, occasionally, when you look at a pedigree you think of that pedigree and a variety of other pedigrees that patients have the same disease, the same phenotype, yet it's due to different genes. This is a phenomenon that we are recognizing more and more often these days in this era of molecular genetics and again this is going to become a rule rather than an exception. A good example is Long QT syndrome. This has both autosomal dominant and recessive forms, there is probably more than one gene responsible for this condition. The recessive form is also associated with general bilateral deafness and this condition is due to abnormal cardiac repolarization identified by prolongation of the QT interval on the EKG and at least four loci are know to cause this

condition. In fact genes have now been identified at these four loci and they are encode ion channels: sodium and potassium channels and the activity of those channels make up the cardiac action potential. So defects in any one of them can lead to this phenotype which is recognized by cardiologists as Long-QT syndrome.

Non-allelic interaction is the concept that genes at two or more loci interact to create a phenotype. Now, this is really extending from Mendelian genetics where one gene causes a disorder, usually. So now, two genes need to be defective in order to create a disorder. One of the best examples is Retinitis Pigmentosa caused by mutations in peripherin/rds and rom-1 genes. But as you can imagine we suspect that a lot of common disorders are due to defects in a series of genes – they are polygenic in their nature. And so defects in a few or even a few dozen loci that interact can create a phenotype. Diabetes, hypertension and so forth are likely to be a variation of this theme of non-allelic interaction.

Anticipation, is another phenomenon that geneticists have pondered about for many years and felt wasn't occurring. This is the concept that within a pedigree the onset occurs earlier and the presentation of a trait is more severe in successive generations. So grandfathers or grandmothers may manifest the disease starting at 50 years of age, have a mild form of the disease, their offspring manifest the disease at 40 and have a moderate phenotype, and their children manifest the disease at 20 or 30 and have the severest form of the condition. Huntington's disease is a perfect example of that – we felt for many years that this was due to bias of ascertainment. In fact, what we were doing is picking up the children with the severe form of the disease – the youngest members of the pedigree, with the severest form of the disease first, and then going backwards and we were missing those individuals that might have a milder form of the disease, and not picking up others in their pedigree, ancestors for example who might have had a more severe form of this condition. But in fact, it's not bias of ascertainment, we know that true anticipation occurs. In fact it's due to unstable triplet repeats that increase in size in successive generations. So they may be of a certain length in one generation and in successive generations they become longer, and they become longer during earlier onset of disease, and more severe forms of the disease. And, three disorders due to unstable triplet repeats are Fragile X, myotonic dystrophy, and low and behold, Huntington disease.

Mosaicism. Now this concept bedevils geneticists because we think in fact that mosaicism is more prevalent than we currently acknowledge. One form is germinal mosaicism in which an individual with a normal gene in somatic cells has gametes that bear both a normal gene and a gene with a new mutation. This new mutation arose during development and it only occurred in the germ line tissue. That male or that female has gametes that carry a mutation or has a normal version of that gene. A good example is Duchenne's muscular dystrophy, 5-7% of mothers of patients with this X-linked disorder actually have germinal mosaicism. And this can masquerade as autosomal recessive inheritance. Because this mom, whom you can test to see if she has a mutation, doesn't manifest the mutation, and yet, she has two affected boys. And, it would appear that for her to have two affected boys, she must be carrying the

mutation somewhere in her tissue. Yet, in fact, the reason that the boys are affected is due to the fact that she has gametes that carry the abnormal gene.

Somatic mosaicism is something that our colleagues in oncology appreciate to a great extent. Individuals with a normal gene in most tissues and normal and mutated genes in some tissues. An example not associated with cancer is McCune-Albright syndrome where this defect in G-protein is a gain of function defect in the G-protein, and the presence of that in certain tissues such as the skin, produces the phenotype. But, it's only present in certain portions of the skin and certain portions of the skeleton. However, many oncologic conditions are due to genes exhibiting somatic mosaicism. In other words, an individual may carry a defect in a particular tumor suppression gene but only the tumor tissue exhibits, now, a defect in both copies of that tumor suppression gene.

Somatic tissue has a defect in one copy and the tumor tissue now has a defect in both copies. And, so, inherited cancers and so the two hit hypothesis put forth by Knudson was that the first hit was an inheritance defect – a recessive inheritance, and then there was a change of somatic tissue defect in a particular tissue in the other copy of that gene and you develop cancer.

And finally, modified genes. Modified genes, here I take a definition from a dictionary, refers to a gene that modifies a phenotypic expression of a non-allelic gene. This is a variation outside of a particular gene that bolsters its expression. And this is believed to be the basis for a considerable amount of variation in phenotypes in Mendelian disorders. So here I show you a pie diagram of the total phenotype of the individual and the contribution of different factors to the phenotype for that individual. Now if this is of a Mendelian disorder, you can imagine that the changes in the disease causing gene are going to be the primary determinant of the phenotype shown here in gray and it encompasses most of that circle. The other genes of the individual inheritance, this is what we will call genetic background would contribute some amount, environment will contribute some amount, a stochastic fate, changes that occur during development will contribute some amount. So this is arbitrary. However you can redraw this diagram for different disorders depending on the influence of the disease causing gene and the influence of the other factors that I show you here. So for this example in ocular cutaneous albinism type 1, the defects in the gene are by far and away the primary cause of variation in this disorder. So there is very little room for other genes in the genetic background to influence the expression of this phenotype. On the other hand in a condition like Marfan syndrome, which we talked about earlier, where there can be varied expressivity, there is a considerable role for other genes, environment, and stochastic factors. In a way, this is shown in the diagram, from a paper by Scriber and Waters a few years ago, in which it was mapped out here on the Y-axis is the role of the environment and stochastical factors and on the X-axis the effect of genotype at other loci and they have plotted out a variety of disorders. As you can see, Tay Sachs is right at one corner here, really the defects in the disease causing gene as was the case with ocular cutaneous albinism is a major determinant of the phenotype. However, other disorders, hemochromatosis for example, you can see is influenced considerably by variations in the environment and stochastic factors and somewhat by the role of genotype at

other loci. My point being that here modified genes and other modifier effects in the environment and so forth have variable effects on Mendelian disorders. And how could these modifier effects affect the expression of a Mendelian trait? They could affect expressivity as I just alluded to in Marfan syndrome, the range of phenotypes associated with a particular genotype under a given set of environmental circumstances. Or they can affect the penetrance, whether or not a particular trait is exhibited and the proportion that would exhibit that trait, again under a set of defined set of environmental circumstances because one would have to control for environment.

So in summary Mendel's laws of inheritance that were derived from peas apply equally well to humans. Inheritance of a trait, much of what we talk about here, diseases in specific proportions within a family are generally due to a single gene defect. If they don't follow specific proportions you can usually infer other forms of inheritance, complex inheritance, mitochondrial inheritance and so forth. Pedigree analysis can suggest the most likely mode of inheritance but I hope that I've given you enough food for thought that there are caveats because there are numerous other mechanisms observed that mask the true nature of inheritance.