

Bob Wildin:

So we'll go ahead and get started with the webinar and the -- we start with an agenda which is relatively focused. This is one in a series of webinars that we hope to give on this topic that gets a little bit more clinical and topical as we go on. So the overall plan for today is to show a case study that really just kind of helps you think about the terminologies and alteration discussions that we're having and then we'll talk about genetic terminology and different types of genetic alterations and modes of inheritance. And then we'll kind of come back to the case study to revisit that and imply it a little bit. So, the case study is about a kiddo named Roger whose a six-year-old boy brought to -- brought by his mother because he's struggling in the first grade. His growth has fallen off and he's the shortest in his class but he's not super short, he's really just at the borderline at third percentile. He's had one seizure early in his life and his head circumference is normal but at the 95th percentile. His mother and father have normal intelligence but report but his father is unemployed due to generalized weakness and pain. His mother is of average stature but his father is 5' 4" tall and is stocky and just for kicks mother is pregnant.

He's had some initial workup other short stature causes like thyroid and growth hormone deficiency and the pediatrician is kind of stumped at this point and wonders if he has an intellectual disability syndrome because of his difficulty in school even though his appearance is basically normal. So a genetics consultant is engaged and detects mild brachydactyly, which is short fingers and borderline upper lower segment and arm span to height ratio. So those are ratios that we measure using a seamstress tape that just has inches or centimeters on it and then we use a little calculator to do that. And these differences indicate mild limb shortness and so the genetics consultant thinks it might be a skeletal -- skeletal dysplasia. There we go. All right. So, a genetic test is ordered. FGFR3, which is the fibroblast growth factor receptor number 3. Gene sequencing is ordered but the ordered test sequences only one exon and a specific exon and it's targeted to detect two variants, C1620C to A and C1620C to G and it does not examine other FGFR3 exons including exon 10 where the fully penetrant pathogenic variant responsible for hypochondroplasia is located. So, the gene test result confirms a heterozygous mutation of variant, p asparagine 540 lysine so the asparagine is changed, at position 540 is changed to lysine. And the diagnosis of hypochondroplasia is made.

All right? So we're going to -- just keep this in the back of your mind as we go forward. So the discussion is a bunch of questions about -- and this is again the preview to keep in your mind. So, the first question is why do the CDNA variants -- and I'm going to get a pointer here I think - - why do the CDNA variants both result in the same protein variant. We'll answer these at the end. How many copies of the hypochondroplasia allele were found? And is this a dominant or recessive disorder? How can Roger's diagnosis possibly help his father? Only some persons with hypochondroplasia have intellectual disability. What two phenomena explain this? And the doctor could have ordered a complete radiographic survey including skull, pelvis, AP and lateral spine, legs, arms, hands, instead of a genetic test to diagnose hypochondroplasia. So, the question is can you give three reasons why she might have chosen the genetic test over the radiographic diagnostic approach? And what did she risk by choosing a genetic test instead of the radiographic approach? Okay. So, let's talk about the human genome which is basically a whole bunch of DNA -- deoxyribonucleic acid which is made up of two anti-parallel strands of a

sugar phosphate background in the blue and base pairs that stick out between them and hook together through hydrogen bonding and the base pairs are specific. So when one base is an adenine that always pairs with a thymine. When it's a cytosine it always pairs with a guanine and so forth.

So, the human genome is all the genetic material in the nucleus plus the mitochondrial genome. We'll talk more about that too. Molecules of DNA that contain the coded instructions for how to build, maintain, and replicate a human being so they're really kind of the blue print. DNA is not identical in anyone but identical twins and it always contains both benign variation and variations that can cause or contribute to disease. And even if they're only recessive diseases we all have some kind of recessive variants there. And it's really big. It's 3.3 billion base pairs if you include everything. So, chromosomes are sort of the packets in which the entire human genome is broken up into. So each chromosome is one strand of really, really long strand of DNA that is rolled up in histones forming nucleosomes and then twisted several times to its condensed state during cell division. It's in that condensed state that we recognize that form or that shape of a chromosome and it has a P arm, which is the short arm and the Q arm, which is the long arm. I always remember that as P for -- if you speak French it's P for petite, which means short in French so that's how I remember it. And when you order a karyotype, which is shown on the left, you get these chromosome pictures with banded chromosomes and there are 23 pairs of chromosomes. One pair from mom, one pair from dad and one pair of sex chromosomes. So 22 pairs of autosomes and one pair of sex chromosomes shown in the blue circle down here. That's the X and the Y in males and XX in females.

So, and the chromosomes always have -- they have a consistent structure, a consistent banding pattern, and they're balanced and I think that's a very important point is we're talking about a balanced -- a normal person has a balanced amount of genetic material and that's really important. All right, the gene is kind of the unitive structure that is encoded in the DNA. SO you have chromosome, which is one long, piece of DNA and within that you have segments which are functional units called genes. And the gene in eukaryotes in humans and animals and plants and things like that actually has sub segments, which are exons that are interspersed between introns. And exons are kind of the business end because they contain primarily the coding material for the proteins. So, when a gene is transcribed and sent out of the nucleus the RNA that results will have the introns cut out and the eventual mRNA will only consist of the exons. Now, I wrote down here that it contains code for proteins. You can have exons early in the gene and late in the gene that don't code for protein portions but they're important for regulation. Interestingly I think that the gene coding regions, if you add up all the exons in the genome they're probably only about one percent of the entire genome.

So, how does this gene get expressed? Some regulator proteins come along usually upstream and in the gene and say, "Okay, this is a gene that needs to be transcribed. It needs to be expressed. It needs to be out there making something that's useful for this particular cell type." And so the DNA is copied to RNA in a process called transcription and again the transcription is like a copying process so that makes sense and it's done by a protein called or a complex called RNA polymerase and you transcribe the backwards part of the DNA because these are complimentary strands and so you get a sense strand of RNA from the anti-sense strand of DNA. And the next step is for that RNA to be exported from the cytoplasm and into the -- I mean

exported from the nucleus, excuse me, into the cytoplasm and actually into the endoplasmic reticulum where this huge machine called the ribosome is taking that RNA and the information encoded in the RNA that was copied from the DNA and turning that into proteins. And it does that by grabbing these transfer RNAs which have specific amino acids tied to their three base pair code and they match that up with the codon in the mRNA and then the amino acid is added to this growing polypeptide chain, which eventually becomes the protein. So we're translating the code in the mRNA to a protein code. And that's sort of described down here on this inset where you have a start codon here and then you have different codons that are coding for different amino acids and we'll talk a little bit more about that.

So the codon UAA is the stop codon and that tells the ribosome I'm done, don't add any more amino acids and let me go. I'm a free protein now. So if the stop codon doesn't show up or if a mutation or a variation results in a stop codon earlier in the gene then that will result in early termination of the protein chain. Okay, let me go back here and just say proteins, you know, what do proteins do. Proteins are really the things that are important in making cells, making them work, and they have a lot of different types of functions. They can be structural elements; they can be enzymes that catalyze biochemical reactions and building other things including proteins that are involved in the ribosome and so forth. They can be proteins that are regulatory factors both inside the cell and outside the cell. They can be receptors, they can be signaling molecules, they can be hormones, et cetera, et cetera. So there's a lot of different ways that proteins can be important but their information is all encoded in the genome. All right. So, let's go on to a little bit different phase where we're talking about some terminology and distinguishing between genotype and phenotype. So, genotype is the genetic code that describes an individual. So if I can describe my human genome and say it has these variants in it that's my genotype.

And the genotype should include information from both copies of the DNA for any particular gene or region. So I got one copy from mom and one copy from my dad so I have two different copies and they don't always have the same variants. They shouldn't in fact and so when you describe a genotype you're describing both copies and it's the entire one. Now you can use the word genotype to describe what's going on in a particular gene and then you're talking about what are the two different alleles? What are the two different sort of cassettes that fit into that gene that were inherited from mom and dad? And the phenotype is the physical manifestation of the genotype in an individual. So we may not know the genotype but we can assess the phenotype. That's what a clinical exam or a radiologic exam or some other general laboratory test may be. That'll tell us something about the phenotype. So the only diagram that I could find that addresses this is one having to do with fruit flies so forgive me for that. But in this slide the phenotypes are normal wings or wrinkled wings and the only ones with the wrinkled wings are the ones that have a homozygous recessive genotype for a small W, which is basically a loss of function mutation, okay? The heterozygous, those with the genotypes of homozygous normal, large W, or heterozygous, large W, small W all have the same phenotype. So you have to understand those differences in how genotype can lead to phenotype.

All right. So, moving on to more important terminology and that has to do with genetic heterogeneity. Heterogeneity means that things are, you know, things are not all homogenous; they're not all the same. So there are different types of heterogeneity. Allelic heterogeneity is

disease that results from different variants in the same gene. So a variant is a -- what we used to call a mutation we now call a variant so that's an A to C or a deletion or something like that. So allelic heterogeneity means you can get the same disease from various different mutations or variants in the same gene. Okay? Locus heterogeneity -- locus is which gene are we talking about here. Is this the NF1 gene or is it the NF2 gene for example? So a particular gene can result in variants from different genes and an example of that is dilated cardiomyopathies. So you can have a dilated cardiomyopathy in a family and you can't distinguish that phenotypically in one family from another family but it could be caused by different genes. So that's an example of locus heterogeneity.

Going back to the allelic heterogeneity an example is hypochondroplasia, which is in our case where most but not all patients with hypochondroplasia have one of two or three different variants in that gene. But there are at least 54 different alleles that have been associated with hypochondroplasia.

Phenotypic heterogeneity means the disease manifestations are different in different people so in the hypochondroplasia case that I mentioned at the beginning the father is of normal intelligence and we're going to presume for the moment that the father actually has hypochondroplasia as well and was never diagnosed and he has no intellectual disability but his son does. So here's another term which is sometimes difficult to capture I think its meaning and that is the expressivity of a gene. So, expressivity or expression is used in different manners. So a disease expression is what the detectable disease manifestations are in an affected individual. So usually that means the phenotype -- what you can kind of see if you've done a thorough physical exam. That's the disease expression so that's the disease features that you see. Some people will extend that and say, "Well, you have to go to a cellular or molecular level." So for example, in sickle cell an individual with the disease may not have a phenotype if they're not having any problems but you may be able to see differences in their hemoglobin or even some sickle cells rolling around in their blood and that would be another example of a disease expression if you look deep enough. So, variable expressivity is affected persons can show different features or difference combinations of features. So just because you have one disease doesn't mean that you're always going to show the same set of features. Some people may show one set of features and other people may show a different set of features.

And that brings me to patterns because even within families where diseases are inherited there may be variations due to unknown factors despite the fact that it's an inherited gene and the gene is all the same in all the members of the family who are affected with that disease. But also among families and that brings up the concept of genotype/phenotype correlations. So different families may have -- may be passing along different variants in that gene and so they have a different genotype. And that may be responsible for the phenotype. So you can begin to -- in some disorders you can begin to say, well, if they have this particular mutation of this particular variant that's causing their disease then they're more likely to have a particular set of features of phenotype than a different variant in that gene. So, here's another term which is important to try to understand and that's penetrance. So penetrance refers to the frequency with which people who have the genotype that is typical of that disease will actually express that disease. So there are some conditions like breast cancer predisposition gene where although you have that predisposition gene you only have say an 80 percent chance of getting breast cancer in your

lifetime. That's a really high chance but that means that there's 20 percent who don't and those 20 percent would be called non-penetrant, okay?

So complete penetrance is everybody with the pathogenic genotype expresses the disease. That's here, okay? Incomplete penetrance is where some, not all will express the disease. And there's a number of sort of patterns that you can see in that. One is lifelong so when you get that genotype at birth, you know; at conception then you may never, ever, ever get that condition, okay? It doesn't matter how old you are or what you're exposed to. It's not going to happen in a fraction of people. The next one is age related penetrance, which is for example, early onset Alzheimer's -- genetic Alzheimer's disease where you don't have expression of the disease until you're older, right? So you're not penetrant until you're of an age where you start being penetrant if that makes any sense. Age related penetrance. And there's also environment dependent penetrance where if you're not exposed to the inciting agent for example, one of the medications or drugs that has a sub population that are particularly susceptible to it for adverse effects you won't be affected. So if you're never exposed to that then you're not expressed. So in the context of the case that I presented I'm just going to read this clip from GeneReviews.org about hypochondroplasia. "Because of evidence that height range and hypochondroplasia may overlap that of normal population individuals with hypochondroplasia may not be recognized as having a skeletal dysplasia. They may not get a diagnosis unless an astute physician recognizes their disproportionate short stature." It's that short-limbed bit that I talked about earlier.

"However, there have been no reports of individuals with an FGFR3 mutation without demonstrable radiograph changes compatible with hypochondroplasia or one of the other phenotypes known to be associated with mutations in the gene." Okay, there are at least 13 different phenotypes associated with mutations in FGFR3. So that's one component. But the point that's being made here is that if you only look at how tall an individual is you will think that hypochondroplasia has incomplete penetrance. If you look at whether they have disproportionate short stature or their limbs are disproportionate in length to their trunk then you will have a higher penetrance level. If you look with x-ray at whether they have a characteristic radiologic findings then that will be a 100 percent penetrant even in a person with normal stature and really not very impressive short limbs. Okay? So I hope that is clear. All right, we had talked about this genetic code before so I'm kind of circling back to that to talk about types of genetic alterations or variants. So, I've used the word variant, I've used the word mutation a couple of times here and the mutation terminology is on the way out. We tend to use variant or pathogenic variant as our standard terminology now and I'll talk a little bit more about that in a minute. So, this is about the genetic code and this is the standard table of genetic code. It is universal. Wherever there's DNA encoding things this is the genetic code that's used.

It relies on a three base pattern, which encodes one amino acid, or the termination, which is stop in here. Okay. It is degenerate which is to say that there are more than one set -- for a number of amino acids -- not all but for a number of them there are multiple codon sets that can encode that single amino acid. So for example, here you can have a codon, which is used -- CU, UCC, UCA, or UCG, which all will encode serine, okay? So you could actually have a mutation or a change in the third position of the codon and not change the amino acid, right? And that's called a synonymous variation, okay? So it doesn't change the amino acid and the protein and so it's much less likely although not certain to cause a problem with that protein, okay? And then there

actually – additionally with serine there are additional ones so you can have a difference in the first position and still encode serine as well, all right? So what this brings up is the fact that if you have a -- let me back up. If the translation of a protein from messenger RNA is reading frame dependent. So if you shift the reading frame the position from each of these bases in the codon then by having an insertion or deletion of a multiple of anything but three then you can shift the frame and everything after that point is translated into different amino acids because the codons are telling the ribosome to add a different amino acid at that position. So this is what's known as a frame shift variant or a frame shift mutation.

All right, so at the DNA level -- so that's kind of the effect of the mutation. At the DNA level here are the different things and different ways that you can mess up a gene. So here is a nice base pair of G AND C and the G is a target of a mutagenic event then you can have a deletion over here where the G is missing and you might end up with a frame shift as I just discussed, okay? You can have an insertion where there's an extra base inserted there, an A in front of the G. And then you obviously because it's scared you get a t on the other strand and you can have a frame shift mutation there for an example. And then you could just get a substitution where there isn't any new, any extra base or missing base, it's just that the G instead of being G is not an A and then on the opposite strand once you replicate that strand it becomes fixed and the opposite strand will be a T of course.

Okay, so those are things that can happen right at the small level. At the macro level on the chromosomes, which these diagrams represent, you can have a deletion meaning a segment that is normally there is missing and you end up with a decreased copy number of those genes that are in that segment. You can have a duplication meaning, and often it's a tandem duplication that's shown here but it doesn't have to be. And you can have an inversion where a segment of DNA is just flipped. And the inversions are less likely to cause problems although you're breaking and reconnecting DNA at two points there and if that interrupts a gene or puts a gene in a place it wasn't before with different regulation or fuses those two genes together then you can still have a problem. Substitution is where something goes in and replaced something else. It's just sort of moving from one chromosome to another. And a translocation is another form of moving something from one chromosome to another. In this case it's reciprocal. The red portion is going on to the chromosome that originally had the green portion on it and the green portion is going on where the red originally was. And the importance of the translocation is whether -- really whether or not it's balanced. If it's an equal swap there's generally very low likelihood of effects. Although when that gets passed on to the next generation the chromosomes may not pair properly and you may end up with an imbalanced chromosome set in an offspring.

Okay, so I'm just going to go over some different types of variants. Base substitution -- one base replaces another. Copy number variants -- a deletion where you've lost a copy. Duplication of triplication where you've gained a copy. So these are called copy number variants. The usual copy number is two except in the male where you're talking about the X chromosome where the usual number is one. And these are called copy number variants or CNV. You'll hear CNV talked about. Repeat number is important because its repeats tend to cause mutations or cause deletions, duplications through a number of genetic mechanisms and they can be tandem meaning they are both oriented -- both repeats are oriented in the same direction. They could be flanking meaning that they're on either side of a region. The flanking ones are ones that tend to

cause deletions like the 22Q11 recurring deletion. They can be a direct orientation repeat or an inverted repeat and the size can be, you know, as large as you want. The whole, you know, the whole chromosome part of a chromosome down to trinucleotide segments. And the trinucleotides are an important class because there's a whole class of genetic disorders such as Huntington's, Fragile-X, and a number of neurologic disorders which are due to trinucleotide repeat expansions. There are also structural variations -- rearrangements and sections of DNA moved around.

That's in a previous slide as well as translocation. I think really the most important take home lesson from this side is that all these different types of variation are not detected by single technologies. So you have to use different laboratory technologies to detect these different types of variations all of which can mess up a gene and cause a disease. So if you're using just one technology to try to understand what's gone on in a genetic disease you may be missing something because there may be variations of different types that disrupt that gene that are not detected by the technology that you're using. Hit that small button up there. All right. The next two slides really are trying to connect the interaction of variation with function. So a gene or a protein has a function. Variation in that may have the potential to cause a difference and that difference may depend upon what environment that variation is found in. And the function can have several different categories, loss of function, [inaudible] function for example in recessives or in haploinsufficiency in dominant disorders. More function, a gain of function, a new function is also called a gain of function or no change at all. So a benign variant is one that we wouldn't have known anything about except we sequenced somebody's gene or genome.

Now, this slide gets into the importance of dosage in this interaction between function and variation. And the -- if the dose is insufficient to give you a normal function for that particular gene then it is a loss of function and that again can be you don't have any -- insufficient meaning you don't have any or insufficient meaning you need two copies worth and you've only got one copy worth and those are recessive and dominant molecular patterns respectively. An excess of function is usually, or a gain of function, for example due to a duplication or triplication of a gene or a mutation that causes an activation of a gene that's normally under regulation and those are usually dominant. A neomorph is something where there's a new mutation -- where the mutation causes say an enzyme which used to convert chemical A to chemical D instead of converting chemical A to chemical C it converts chemical C to chemical D now and B happens to be oncogenic for example. So that's a neomorph. And then if there's just enough then it's benign. So that's sort of what the balance is about that I was talking about earlier. Okay. Modes of inheritance. We can figure out modes of inheritance in several ways. We can infer them from a pedigree, your family history tree. We can predict them from the functional affect of a pathogenic variant that I was just talking about and we may need in that process to correct for lethality. So for example if a dominant disorder is lethal in young life it's not going to be seen in a family in a transmitted fashion because it's not transmitted on because the person who had it died before they could pass it on.

Also you need to think about whether it's a germ line typical inherited disease pattern or whether it's somatic mutations of variations that are found in tumors. So obviously if it's found in a tumor and it's not in the regular DNA of the person then the inheritance pattern is kind of irrelevant. Okay? So the different, let's start with the dominant inheritance pattern and the

diagram here is a pedigree or a family health history and the affected individuals are marked by being darkened here and of course boys are squares and girls are females and the oldest generation is at the top, the youngest generation towards the bottom. So the characteristics of dominant inheritance are that affected individuals affect both sexes equally on average and if you know what is going on at the genotype level one of the two alleles of the diseased gene is bad. In an unaffected individual there's no disease allele so neither copy of the disease gene is bad and since they don't have a bad copy they can't pass it on. It doesn't -- it isn't transmitted from one generation to the next. So for example this guy here isn't affected, doesn't have the gene, and has all kids who don't have that disorder. Another characteristic of dominant inheritance is a vertical pattern, which means that you see this in multiple generations and you don't see a lot of generations skipping. It tends to be passed down from one generation to the next. And on average an affected person will have a 50-50 chance of transmitting it so 50 percent of the people who are at risk of inheriting the disease will get it on average.

With autosomal recessive inheritance some people talk about this as having two bad copies of the gene. You get one bad copy from mom and one bad copy from dad. But from a functional standpoint really the operational definition is that you don't have a normal copy. You don't have a backup copy, okay? That's good; that can provide this function that's missing in the mutated or variant allele, okay? In an unaffected individual you will have at least one normal copy. So that's the criteria for being unaffected is that you have a normal copy of that gene. In a carrier, the carriers are generally unaffected in recessive disorders and they have a 50-50 chance of transmitting. There's a couple of diagrammatic representations of being a carrier and this slide the dot is what is used to represent a carrier for a particular disorder. The last characteristic is that both parents of an affected person are carriers or they could be affected but that's pretty rare. So this guy down here is the affected person in this pedigree and by definition both of his biologic parents -- not his adopted parents but his biologic parents are carriers. And so those are the primary characteristics of autosomal recessive inheritance. So let's talk about another recessive, X-linked recessive and remind you that the X chromosome is the strange beast that's present in two copies in females, in one copy in males because males have instead this tiny, little guy called the Y chromosome, which is important for making the male. So, an affected individual with X-linked recessive inheritance has no normal copies, are generally males because the male with an X passes on only his X to his daughters and the X is mutated or abnormal. All of his daughters will be carriers because he passes on his Y to make his sons.

All the sons are unaffected and can't pass on the disorder and here I have -- and males are affected but rarely females. That's a more complex subject we can go to in the Q&A if you want to. But the important point is you cannot absolutely rule out some X-linked recessive disorder expressing themselves in females. The unaffected people have at least one copy same as in the autosomal recessive. They are the non-carrier males and most females, right? The carriers are typically unaffected but there can be several manifestations in some disorders and these are the females or males who have an extra back up copy. So males with Klinefelter syndrome or XXY, all right? And these individuals will transmit -- the females will transmit at a 50/50 ratio. The mother of an affected person, so remember in autosomal recessive both parents of affected. In X-linked recessive the mother is an affected. The mother of the affected is a carrier but not always and that's a real little tricky thing. So if it's a benign condition like I have X-linked colorblindness and so I'm always going to inherit that from my mother and my mother's always



going to be a carrier for that. When the affected males are such that the affected status prevents them from reproducing then the population dynamics results in a high frequency of new mutations and so you can predict the mother will have two out of three -- two out of three of the mothers will be a carrier but one of three of them won't be so you may actually have to test mother to determine whether or not she's a carrier in X-linked recessive disorders. All right, how about Y-linked? There are some y-linked disorders. They're quite unusual because most of what's on the Y chromosome is junk, okay? There is the male determining region and then a few things and then a few genes that are on the very tip of the Y chromosome that are also on the very tip of the X chromosome that are called the pseudo autosomal regions because they are copied on both and act just like autosomes. All right, mitochondrial inheritance -- and I think we're getting to the end here -- is in both sexes are affected. There's a lot of variable expression. We're talking about inheritance of the small, round circle of DNA, which is intrinsic to the mitochondria and encodes some of the mitochondrial genes. Others are encoded in the nucleus of the cell and are migrated into the mitochondria. It has also vertical transmission but its maternal lineage only and never transmitted from males because the mitochondria are only transmitted via the egg, never via the sperm. And the other way you can notice mitochondrial inheritance is that the phenotype deals with the energy intensive organs because the mitochondria -- mitochondrial deficiency is mitochondria energy producers for the cell and cells that consume a lot of energy are the ones that are going to be sick fastest. And then the last sort of pattern of mutation is de novo or new mutation, new variation in the family.

There's no family history in the dominant situation. It's not present in the DNA of either parent when you go looking and it's generally considered to be supporting evidence that the variant that is new could be pathogenic if it's associated with a disease. It's not iron clad but it is supported evidence. So, I'm going to come back to the case study and just summarize this so Roger is a short, slow kiddo with a bigger head. He has a family history where his father has some weakness and pain and is a bit short. He had a genetics consult which found some phenotypic differences that suggested a skeletal dysplasia. And a genetic test was done that showed a heterozygous variant that predicted a protein difference of asparagine 540 to lysine. So we'll go through now the questions if I can get that click to work. Why do the C variants C1620, C to A and C1620 C to E both result in the same protein variant? How many copies of the hypochondroplasia variant alleles were found? How can Roger's diagnosis help his father? Only some persons with hypochondroplasia have intellectual disability and why did the doctor choose this particular approach? So the answers are that the CDNA -- I'm sorry that these two variants occur at the same position, one going from C to A and the other from C to G and as we talked about earlier you can get an asparagine to lysine by going from C to A or C to G due to the degenerate codon in the genetic code.

How many copies of hypochondroplasia variant allele were found? Is this a dominant recessive disorder? So there's one variant allele and one normal allele in the ATBP binding segment of the FGFR3 tyrosine kinase domain. So the test result was heterozygous for the disease-associated variant. And there's one disease associated variant and that's compared with a normal reference sequence. Hypochondroplasia is a dominant disorder both by inference from pedigrees and by the biologic basis, which is a constitutive activation of the receptor tyrosine kinase, a gain of function. Okay, how can Roger's diagnosis possibly help his father? His father's short stature and stocky build suggest that Roger may have inherited hypochondroplasia

from him. A significantly increased incidence of spinal stenosis and bony compression occurs in this disorder. So Roger's diagnosis might lead to diagnosis in the father and detection of and surgery for spinal stenosis. So Roger's father might recover from this pain and disability, which would be a really great thing. I put this in here as an example of how genetic testing is in some ways, in one very important way very different from regular kinds of laboratory testing and that is it has potentially important carry-on implications for other family members so its value actually is not just to the patient but to the entire family.

So, the next question was only some persons with hypochondroplasia have intellectual disability and what two phenomena explain this? One would be variable expressivity. So we did talk about that so that's where one individual with the disorder may have a certain pattern of expression, a certain pattern of features whereas another individual even in the same family may not have the same pattern. So that's variable expressivity. It makes it difficult sometimes to determine when you take a family history whether there's really something going on in the family that's dominant or not because they have different manifestations. So for example in a BRCA family you may have people with breast cancer but other people with ovarian cancer only. And in the early days we didn't recognize that those were part of the same disorder and so we didn't recognize that they needed to be counted together in terms of figuring out whether this was a dominant at risk-inherited family. So the other type -- thing -- phenomenon is genotype/phenotype correlation and we mentioned that. So the particular variant, disease associated variant in this case of hypochondroplasia is associated with a higher incidence of intellectual disability. Not 100 percent but a higher incidence of intellectual disability than some other variants that also cause hypochondroplasia.

All right, so the last one was about why didn't he -- why didn't the doctor order all these x-rays instead? And the answer is that the complete -- this whole complete radiologic survey is necessary to diagnose hypochondroplasia and the radiation exposure is significant and even then radiologic diagnosis can be difficult and depending on the experience of the radiologist [inaudible]. So, I'm getting the signal I need to wrap it up. So the second point is that gene test is less expensive -- \$200-\$300 for a single exon and the test for a variant is associated with intellectual disability. So if we find this variant then we believe we've explained his intellectual disability and we don't have to look deeper to try to explain it and say, "Well, he had the skeletal dysplasia but we haven't still explained his intellectual disability. We can probably stop there. So that's a plus. So I'm going to move on because we need to get to questions and so I'll thank you for your attention and for your patience and we are really interested in your feedback since we're starting this as a pilot and to see how useful this kind of thing is and whether there are changes in the format or the approach that can be useful to you guys. And these are just some resources that I used in the case study development. And I'll stop there.

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