[BRIGHT MUSIC]

JONATHAN ALDER: Thank you for that introduction.

I'm going to lead off this triple header today and give a little bit of background on the unique genetics of telomeres and how they relate to lung disease.

So here are my disclosures. I have none relevant to this. I am a telomere biologist. I see everything through the perspective of telomeres.

[CHUCKLING]

So this is a human metaphase spread. The DNA is stained blue in chromosomes, and in red is FISH or fluorescent in situ hybridization for the telomeres. If you think of DNA as the instructions for life, then you can think of telomeres at the beginning and the end of the instructions of life. They form caps on the end of each chromosome. They are composed of a simple nucleotide repeat, TTAGGG, repeated many thousands of times at the end of each chromosome.

They serve one essential role, and that is to suppress the DNA damage response. So the end of each chromosome appears identical to a broken piece of DNA and should trigger a DNA damage response. However, that process is blocked by a group of proteins that bind the telomere sequence. There are six proteins, termed shelterin, that coat telomeres and prevent them being recognized as broken pieces of DNA. If telomeres become too short or you lose any of the sheltering components, they are recognized as a broken piece of DNA and trigger a DNA damage response that can culminate either in cellular senescence or apoptosis.

Telomeres face a unique challenge, and that's something that's termed the end-replication problem. James Watson, in 1972, while working out the machinery responsible for DNA replication, recognized that there was no mechanism for copying the 5'-end end of DNA, and recurrent rounds of DNA replication would lead to eventual loss of DNA. This process, if left unchecked, would eventually erode all of our DNA.

However, a remarkable enzyme called telomerase reverses this process. It's composed of a reverse transcriptase, the only endogenous reverse transcriptase in our genome, that is, it converts RNA back to DNA. Also, we encode our own telomerase template to be reverse transcribed into telomere, called tRNA for telomerase RNA, and these two together act to extend telomeres.

Now, telomerase expression is limited to a very limited number of cells within the body. There are also a number of accessory factors that I'm going to mention later in the talk, including DKC1, which is necessary for telomerase activity.

In recent years, mutations in a number of genes that are involved in telomere maintenance have been identified in a number of diseases. Early mutations were identified in dyskerin. Later on, mutations were identified in telomerase RNA, and later in the reverse transcriptase component. These underlined a unique and rare disease called dyskeratosis congenita, which I'll say more about.

Since that time, mutations in a number of additional genes, including the CST Complex, composed of CTC1, STEN1, TEN1, and a number of other genes, have been identified and cause telomere-mediated disease.

Interestingly, and relevant to the talk today, the most recent three genes that have been identified that are responsible or necessary for telomere maintenance, RTEL1, PARN, and NAF1, were all identified studying lung disease. So there is a unique link that we'll talk about.

Mutations in genes responsible for telomere maintenance cause a one of a kind genetics and inheritance pattern. Because it is the telomere length that drives the phenotypes we're going to talk about today, each generation that a mutation is carried results in shorter telomeres being passed on in the next generation. This is known as genetic anticipation and manifests as disease presenting earlier in life. And unique to the telomere syndromes is a changing phenotype or clinical presentation, such that early generations typically develop lung disease or idiopathic pulmonary fibrosis and subsequent generations often develop liver disease and finally bone marrow failure, such that an individual pedigree can be seen at three different services within a single hospital. You may have pulmonary, liver clinic, and bone marrow failure syndrome in the children's hospital, so truly a unique challenge.

Telomere length can be measured clinically. Shown here is some data from an assay called Flow-FISH that combines flow cytometry and fluorescent in situ hybridization to estimate the telomere length from peripheral blood cells. Shown here, these lines represent a nomogram of a reference population of telomere lengths. The dots represent 100 patients with different mutations in telomere maintenance genes, and I want to draw your attention to this apparent line that appears where people show up with clinical phenotypes. For the most part it's not the gene that matters, but the telomere length that is what drives phenotype.

You can see that one of the issues we face when studying telomeres is, the population approaches this critical level as we age, such that distinguishing between individuals with short telomeres and normal is easy in children. As we approach the sixth and seventh decade, these groups overlap.

And this is just to illustrate that in general, individual genes do not cause different telomere lengths. The notable exception is DKC1. It's found on the X chromosome, so individuals with mutants are effectively null.

I mentioned before, and I just want to illustrate this, that telomeres cause this very unique age-dependent clinical presentation, where people present different phenotypes depending on the age at which they reach this critical telomere length. Children typically present with primary immunodeficiency, young adults can present with bone marrow failure, in the fourth and fifth decade they often present with liver disease, and later in life primarily as lung disease, either emphysema or idiopathic pulmonary fibrosis.

I want to point out that by far the most common clinical manifestation of telomere syndrome is idiopathic pulmonary fibrosis. This is a disease that affects about 50,000 people in the United States. It's a progressive scarring of the lung. Average lifespan after diagnosis is 2 and 1/2 to three years. It was historically thought to be primarily caused by environmental exposure, and importantly, lung transplantation is the only life-prolonging phenotype.

If you look specifically at idiopathic pulmonary fibrosis alone, over 30% of the familial cases are caused by mutations in telomere maintenance genes. If you look at sporadic cases, somewhere between 9 and 11% of sporadic cases of idiopathic pulmonary fibrosis are caused by mutations in telomere maintenance genes.

I wanted to show this figure from a recent publication from Genentech where they measured telomeres in approximately 10,000 individuals with age-associated diseases, including Alzheimer's disease, Parkinson's, macular degeneration. And interestingly, of all the age-associated diseases, only one is associated with short telomeres, and that is IPF.

So here at the University of Pittsburgh in UPMC, we sought to characterize a cohort of patients that had undergone lung transplantation here. As a retrospective analysis, patients were selected based on a family history or some other clinical indication. Whole genome sequencing was carried out at the UPMC Genome Center, and genes associated with telomere biology were analyzed. Close to 50% of the patients sequenced, we identified rare variants in telomere maintenance genes at first pass.

I want to emphasize this is the low-hanging fruit. We are still undergoing analysis of the non-coding regions of these genes, structural variants and copy number variants. But you can see we found a host of rare variants in these.

Some of them have been previously reported I'm going to call your attention to one very unique mutation in this individual here. A 43-year-old male-- that's quite young-- had a mutation in the RNA component of telomerase.

So just briefly, this was a unique mutation because it occurred within the template sequence that is reverse transcribed into telomere sequence. Typically, this sequence beginning right here, TTAGGG, would be reverse transcribed for a new telomere. This mutation is predicted to disrupt that sequence. And indeed, because we had whole genome sequenced, we noted that this patient had a variant telomere sequence at the end of each of its chromosomes. I've shaded in blue the canonical sequence for telomeres. And this perhaps one of a kind mutation introduces a variant telomere sequence in this patient.

So this was previously thought to be highly toxic, in fact has been proposed as a method to fight cancer, was introducing mutations into telomerase RNA. But this data suggests that there are some mutations that are well tolerated.

In conclusion, I just want to state that telomere genetics really are unique and present a multidisciplinary challenge. The pathology is driven by telomere length, not specific mutations. Lung disease really is the most frequent, but there is a unique connection between the lung and telomere length.

To move forward in understanding and more completely understanding, I really think that whole genome sequencing is essential. We're really good at identifying variants in the coding sequence. It's time to move into the non-coding sequences to understand them.

And finally, given the clinical implications that are going to be discussed by the following speakers, in my opinion, genetics should not be ignored in the context of lung disease. The guidelines from the field typically are, do not measure telomere lengths and do not do any genetic testing and transplant them anyway. But I think given what we know now and what we're going to talk about, there's good reason to include genetic testing or telomere length measurement in this evaluation.

Let me just acknowledge those who contributed and helped complete this work, from my lab, the University of Pittsburgh and the Genome Center, and funding from multiple groups. Thank you.

[APPLAUSE]