[MUSIC PLAYING]

MARK RUSSELL: I'm Mark Russell. I'm a pediatric cardiologist here at the University of Michigan. And this is my first time at this conference, and I'm really excited to be here.

So I wanted to share a few thoughts on congenital heart disease and neurodevelopmental outcomes, and what we've been learning in terms of the role of genetic factors, and also touch briefly on biomarkers, as well.

So how do genetic factors influence our measured neurodevelopmental outcomes? I think of neurodevelopmental outcomes as a combination of both brain injury and brain development and how these different factors affect both how the brain develops and then how it responds to an injury process.

And genetic factors can influence any of those factors, including hemodynamic factors, oxidative stress, and how the body responds to these different stresses on the brain. Much of what we'll be talking about is how genetic factors affect brain development and how there are shared developmental factors that affect both the brain and the heart.

So the first thing I'd like to talk about is what's called copy number variations. These are duplications and deletions that affect large parts of the chromosomes. The most examples of that would include Down syndrome, which is a duplicated whole chromosome, and DiGeorge syndrome, which is the deletion of a small part of chromosome 22. And these can have big effects on both how the individual develops and how the heart and brain develop.

So deletions and duplications-- so this is just a slide showing what that looks like. So there's a area on the chromosome that gets tandemly duplicated. Usually there's an area just before it and just after it that looks somewhat alike. And so the replication machinery kind of loses track and duplicates that segment.

Or inversely, it can skip that segment altogether resulting in a deletion of that segment. And if it's a small deletion, that can be innocent. Everybody in this room has small deletions and duplications. The pathologic ones tend to be the ones that are larger, and the ones that haven't been seen before in other patients, or are not present in the parents. So sometimes we'll actually look at the parents to see if they share that same deletion or duplication.

So how do these affect neurodevelopmental outcomes? So deletions and duplications lead to what are called copy number variations in the number of genes. It can affect either one gene or several genes resulting in gene dosage effects-- so either too much of a gene or too little.

Deletions and duplications that are large-- half a million base pairs or so-- occur in about 10% to 15% of our patients with critical congenital heart defects. They are also commonly noted in patients with cognitive and behavioral deficits. So if you look through the neurodevelopmental literature, these are likewise quite common.

And so one of the things that the cardiologists want to know is to what extent can we attribute poor cognitive and behavioral outcomes to these chromosomal defects. And so we're looking at the whole congenital heart disease population, and singling out those that have these chromosomal defects, and comparing them to the population as a whole. How do these patients with these chromosomal defects do to the congenital heart disease population as a whole?

And this just shows a study that was done by Bruce Gelb in collaboration with the Pediatric Heart Network that looks at defective copy number variants on outcomes for infants with single ventricle heart defects. And it showed that significant-sized deletions and duplications occurred in about 14% of the patients.

And those patients-- compared to the CHD population as a whole-- showed worse linear growth and impaired neurodevelopment with a diminished PDI score on the Bayley's compared to the rest of the CHD population. So these seem to be occurring in a subset of the population and having a significant effect on both growth and on neurodevelopmental outcomes.

So just to summarize this portion of it-- so CNVs occur in 10% to 15% of patients with congenital heart disease. They do significantly affect neurodevelopmental outcomes. And they're going to be important to consider when looking at our outcomes in assessing different strategies to help improve outcomes.

But it's not the whole story, as it only-- as I said-- only affects a small subset of patients that we see are significant. But they're still-- the majority of patients wouldn't fall into this category. So we need to look for other genetic causes that are maybe affecting heart development or brain development.

So then the next thing we wanted to look at-- we were talking about big things that affect big areas of the chromosomes. So now we want to look at single gene defects. So if we interrupt the function of a single gene, what effect does that have on both heart development and brain development?

And I threw up there a slide of a patient with Noonan syndrome just to remind us that defect in a single gene can lead to abnormal development of multiple organs and tissues, craniofacial structures, other developmental abnormalities, including neurodevelopmental abnormalities.

So I'd like to introduce a pediatric cardiovascular genomics consortium, which is a multicenter exome sequencing effort supported by the NIH. They looked at multiple CHD subtypes including conotruncal defects, AVSDs, left ventricular outflow tract obstructions including hypoplastic left heart syndrome, ASDs and heterotaxies.

And at the time of the paper that I'm going to discuss, they'd sequenced about 1,200 trios. I think that number is now up to about 12,000. Is that-- 2,800, OK. So they're continuing to add to that almost on a daily basis.

They've been focused on de novo DNA sequencing and variants. And de novo variants are ones that don't occur in either parent. With the thought that if it's a new incidence of heart disease in the family-- neither parent has heart disease-- and now suddenly you have a new mutation in the child, and they have heart disease, that it's more likely to be associated with the cardiac disorder.

And they found that there was about a little over one new de novo genetic variant per child in patients with congenital heart disease. And that wasn't very different from control. However, what they did notice is that more variants-- more of these genetic variants-- fell into the realm of what we call damaging variants.

So there are some genetic variants that are innocent or don't change the protein very much, if at all. But in patients with congenital heart defects, the mutations are more likely to be damaging. They are also much more likely to involve genes that are involved with heart development or other developmental genes that were critical to how the fetus develops.

So looking at damaging de novo mutations associated with congenital heart disease and neurodevelopmental outcomes. Subjects with congenital heart defects were enriched for loss of function or very damaging mutations in genes expressed in heart development. And they were very enriched in subjects who not only had defects of heart development but also had other congenital anomalies and neurodevelopmental deficits.

And if you put all three together-- so with CHD compared to controls-- the defects are much more likely to occur in a heart-development gene. If you looked at just isolated CHD versus CHD plus another developmental defect, much greater enrichment of mutations within these heart-development genes.

And then if you split it out with neurodevelopmental defects only, other congenital anomalies only, or both, by far the greatest enrichment for damaging mutations in heart-development genes occurred in those that not only had a heart defect but also had neurodevelopmental defects and other congenital anomalies.

And this just shows one of the papers that came out of their work looking at de novo mutations in congenital heart disease with patients with both neurodevelopmental and other congenital anomalies. Again, showing that a single gene mutation could lead to multiple developmental defects, including defects in both heart and brain development.

So the progress to date-- just to go back to our scheme of the contributors to neurodevelopmental outcomes. So what we've been learning about-- for the most part-- is how the genetics impacts brain developmental process. And so then I'd like to talk a little bit more about the next phase, which is looking at how genetic defects or genetic variation can affect brain injury and response to brain injury. And included in this will be pharmacogenetics, although we don't have a lot of data on that yet.

So genetic factors and how genetic factors affect different outcomes. So on the y-axis is the impact that the genetic factor will have on outcomes. And on the x-axis is how frequently those variations are seen in the population. So what we've been talking about so far is this area up here, which is rare variants that have a very high effect on how development occurs.

But there's also very common variants. What is affecting the vast majority of our patients? And these are much more likely to be of low effect individually, but may have a very great effect when combined. And so how to tease out what some of these more common variants are doing that may have a lower individual effect but a important effect when they're combined within a patient.

So one of the important pieces of work that I wanted to mention was a work that was pioneered by Bill Gayner looking at ApoE. And ApoE is a protein-- or is encoded by a gene on chromosome 19. There's three major isoforms-- ApoE2, ApoE3, and E4. So what he's showed over the years is that-- and what he recently replicated-- was that there was an association of apolipoprotein E epsilon 2 allele with neurodevelopmental dysfunction after cardiac surgery in neonates and infants.

And this was a kind of important finding. Because this genetic association study is going to be hard to replicate because of-- as I said-- they're common variants but a very low effect in individuals. But what we've learned is that ApoE really has an important role in survival of neurons and regeneration of neurons. So it's thought that this variation is affecting the resiliency of the brain to injury that may occur during the bypass process, or other developmental processes, or surgical processes.

So we also want to look at common variants and how they affect neuro-injury. So ApoE affects how resistant the brain is. We also want to look at the injury process itself. And there's increasing literature on the effect of anesthetics on brain injury. Volatile anesthetics may enhance a hypoxic-ischemic injury. Other medications, including dexmedetomidine, may have a somewhat protective effect. And that's something that needs to be further studied.

There may be an important role for genetic variation in drug metabolism pathways. The Body hasn't been-- evolution hasn't really considered how it's going to respond to different medications. So there could be very, very different broad range of how the body responds between individuals to these different anesthetic agents. And there's a big role for understanding the pharmacogenetics of how these drugs are processed and metabolized and what their effects are on the developing brain.

This just shows the pathway for once a drug is-- how it's processed once it's in the body and the different processing and elimination. All of those will affect-- the effects will affect both the efficacy of the drug and the toxicity of the drug. And one of the focuses has been on the cytochrome P450 pathway, which is with major drug metabolism pathway. And so the hope is in the future we'll be able to read somebody's DNA and come up with a precise prescription of treatments and drug exposures that will lessen their risk for neurologic injury.

So this is kind of who's at risk. How can we identify the vulnerable patient? Also, once the injury has occurred, how can we detect it? So how can we detect neural injury? And what are some of the biomarkers we can use to assess whether injury has occurred?

So if you look at the injury process, the injured cell will-- or the dying cell will-- release proteins and small RNA molecules into the extracellular fluid. These will then become in equilibrium with the cerebral spinal fluid. In some cases, if there is a breakdown of the blood-brain barrier, these will communicate directly with the systemic circulation.

So in order to detect them, since we don't have CSF monitors, they have to find some way into the systemic circulation. And this can either be through equilibrium or through breakdown of the blood-brain barrier, either temporarily or more permanently. And then these present in the urine or the bloodstream and can be then detected and validated as biomarkers of neurologic injury.

But the one thing I would like to point out is that these proteins and microRNAs can be released at different time points. And also how quickly they get into the blood will depend on different factors about the integrity of the blood-brain barrier. So there's knowing when best-- when to test for these different biomarkers can be quite a challenge.

The biomarkers-- as I said-- are proteins related to the different neuronal cells. And a lot of the work has been done with both S100B and GFAP, which is a glial protein. The other biomarker of neurologic cell injury, that's neuron-specific enolase MicroRNAs are small RNAs that are regulatory molecules. These are just beginning to be more extensively studied. And not much is known about them, but they hold exciting interest as a novel way of tracking neurologic injury. And keeping in mind that these all must cross the blood-brain barrier to be detected, so that's one of the challenges.

So biomarkers and neurologic injury. Serum biomarkers, including S100B and neuron-specific enolase, have correlated with neurologic injury in many adult diseases-- including ischemic stroke, traumatic brain injury-- after cardiac arrest and after bypass. The S100B increases within the first 24 hours after bypass in adult and peds

And correlates with transient neurologic decline in adults has not been definitively shown to be associated with neurologic outcomes in pediatrics. Although, there is some data to suggest that later-- if you check it at later time points-- that there may be some correlation. Again, that has to do with the release and how things are processed within the blood-brain barrier.

And then neuron-specific enolase also increases within the first 24 hours. Correlates with transient cognitive decline in adults but not correlated with neurologic outcomes in peds yet. And the one benefit that the adults have-- that we don't often have-- is that they're able to do sensitive pre- and post-testing. And for a lot of the infants we have, we just don't have a sensitive way of doing cognitive testing in the infants. So we may need to do more validation with imaging-- such as MRI-- to correlate with some of these biomarkers.

So microRNAs-- as I mentioned briefly-- these are small, about 22 nucleotide, noncoding RNA molecules that regulate gene expression and involved in a wide range of regulatory roles. And they vary by cell type and may prove a more specific indicator of damage from two neurologic cells.

So in summary, neurologic deficits-developmental deficits can occur with all CHDs-- more common if there are also other developmental deficits, including other craniofacial abnormalities or other abnormalities of other organs and tissues. Chromosomal abnormalities, such as deletions and duplications, lead to significant neurodevelopmental deficits in approximately 10% to 15% of our patients.

Rare single-gene mutations can lead to abnormal heart and brain development resulting in both CHD and neurodevelopmental deficits. And common genetic variation leads to small but important differences in ND outcomes across patients due to differences in neuroprotection-neuroresiliency. And neurodevelopmental defects can be thought of as a combination or intersection of developmental defects and neuro-injury pathways.

So in further summary, improvement in neurodevelopmental outcomes may depend on linking genetic data to standardized and well-documented neurodevelopmental outcome data to define genetic signatures associated with adverse outcomes. From that, we can identify high-risk patients and target vulnerable pathways. We can also identify biomarkers that detect early neurologic injury before it progresses to irreversible deficits. And I put the target up there just to indicate that what we're trying for is targeting our therapy to the different subpopulations of the patients that we encounter.

That's it-- and, oh, my thank yous. So I'd like to think the organizing committee for inviting me. And I'd like to also thank my wonderful collaborators including Bill, Nicole Wilder-- who are both here today-- Tom Miller, as well, and the PHN, and the PCGC.