

Disclaimer: These are notes typed by Ruth Dameron while listening to a presentation by Dr. Nicholas (Nico) Katsanis re his genetic research into BBS. There is no guarantee that this is an accurate representation of what Dr. Katsanis said. Do not quote these notes and attribute the content to Dr. Katsanis!

Dr. Lewis “holds Nico in *extremely* high regard” – “both his intelligence and curiosity”.

Dr. Nicholas (Nico) Katsanis is Associate Professor at Institute for Genetic Medicine at Johns Hopkins.

Genetics of BBS

- What’s new?
- How is the BBS puzzle fitting together?

Dr. Katsanis said, “I am very grateful to put human faces on what I do in my work of genetics which is primarily moving colorless liquid from one tube to another!”

The goals of his research:

1. Molecular prognosis of the disorder
2. Clinical prognosis of the progression
3. Identification of the cellular defect
4. Selective intervention.
 - a. blindness
 - b. kidney problems
 - c. weight management

We can’t just treat symptomatically – need to treat before the symptoms happen and that’s genetics.

The rate at which eyesight loss happens is vastly different – need to be able to understand and quantify so we can treat, prepare, and be informed re future.

Without quantitative knowledge of the cellular defect, we can’t get rid of it. What makes it more complicated, every tissue and every organ in a BBS patient has some problem or another.

I think about it in very long term – 20 to 30 years – like space program timetables.

I don’t believe there will be a magic problem to relieve all the problems of BBS

We need to work on this tissue by tissue, system by system. We need to focus first on the more easily accessible and less on those more intractable.

Halting the loss of retinal cells is a major goal but I think it will be one of the most difficult.

I think we can deal with the renal defects the soonest.

One of the things is we have a pretty good understanding of is the cellular level of kidney defects

We can access the kidney with drugs because the kidney’s role is to filter and concentrate. So we can give a weak dose of something and, by the time it gets to the kidney, it will be in a strong enough concentration to do the necessary job.

In the kidney, we chanced upon a signalling pathway for which there have been thousands of studies done.

Nico's bias:

1. get the genes
2. understand the relationship between the mutations in the genes and the disease
3. understand the normal function of the genes/proteins
4. correlate mutations with cellular dysfunction
5. develop therapeutic targets – been able to start this for one cell type, one tissue

Focus on which cells we will study, understand it there, correct it there, and then move on.

History and progress:

From 1994 to 2006 – in 1997, I thought it was a single gene disorder

The complexity turned out to be a blessing in disguise. We now have up to 12 BBS genes which gives us 12 entry points into the disease.

The genes do not produce 12 varieties. Rather, we have 12 proteins that create the same cellular defects. A group of people who each have a recessive pair of the same BBS gene can present with all manner of variety in symptoms. But someone who is affected more severely has multiple types of BBS genes.

1999 – “we knew nothing”

To do genetics, you need people who are willing to donate blood, histories, etc.

Since then, we have identified 12 genes in seven years.

This is slowing down – we are getting very close to having found ALL the genes that can cause BBS.

Change in understanding:

until 1994 – thought BBS all caused by one gene; now we expect 25 or 30 but we have already found half of them in seven years.

until 2001, thought BBS is a monogenic disorder

Now we know it can take 2 or more different genes to get BBS to modulate and vary the disorder.

from 2001 to 2003 – thought the second type of BBS gene gave rise to BBS (There was a family with two sons who both had a recessive pair of the same BBS gene. One had the syndrome; one did not.

The one who had the syndrome also had another type of BBS gene.)

Now we know having a second or third type of BBS gene can sometimes affect the severity of the disorder

Challenge now in 2006:

Be able to establish the precise impact of the gene mutation to be able to practice predictive medicine

BBS8: first insight into function

- one family with BBS8 mutations has situs inversus – flip of the axis of symmetry of the body – you're normal but your organs are on the opposite side of the body than expected, etc. – we KNEW that was a defect of cilia.
- left-right axis of symmetry is a defect in CILIA – this was a huge breakthrough

Cilia are whip-like protrusions that exist on the surface of most cells of the body.

Defects of cilia and their anchoring structure are at the root of all the problems. In each system, organ, and cell type, you get a unique set of symptoms because of the role that cilia play in that system or organ.

Flatworms – we have created groups of flatworms with different types of BBS.

Flatworms are a powerful system for the study of cilia. They only have 959 cells. (Yes, someone counted them!) Each cell has been characterized in detail. 60 cells are ciliated nerve cells. Neurons that have a cilium at the tip. These are the neurons with which it communicates with its external environment. Find food (they like alcohols!), recognize when something is toxic, find worms of the opposite sex.

The cilia in the worm mediates all dysfunctions. Can paint cilia with fluorescent dyes. We were able to use these flatworms to show that for each BBS protein (gene) each was found specifically on the worm's cilia.

In human cells, there are cilia in EACH cell. All the BBS proteins localize and regulate the traffic of signals along the cells.

What do cilia have to do with BBS symptoms? Here are some examples:

1. Eyes – transport of proteins to the retina. The cilium is the toll bridge. The transport across the cilium does not work well, buildup of protein at the bottom of the cell, eventually becomes toxic to the cell and the cell dies. Challenge: Acts like a closure of a lane on a hwy. Find a way to “open up the other lane”. We probably can never reverse the damage that has already been done. We may slow down the time it takes for the cell to die and may be able to prevent further cells from dying. This is extremely difficult and extremely long term.

2. Kidneys – cilia sense salts and pressure; this role is critical for development and function.

Critical for dev of organ, function of the organ, and regeneration of the organ.

They have nothing to do with regulating flow of urine but they sense what is going on outside the cell and tell the cell what is happening so the cell responds appropriately. Misinterpretation of the information especially during development causes the kidney problems.

If we can link the BBS causes to other research groups' kidney issues, then we can get funding. For example, if what is happening in the kidney is the same as what is happening with another more common disease such as polycystic kidney disease, then we are more likely to get support.

Other systems affected as well:

Nervous system: nerve cells transport proteins from the center to the tip like cilia

Fingers/toes: defects in “sensing” position might lead to polydactyly. Cells in the forming digit have a natural ability to sense their relative position to end up in right place. Cilium is essential to translate the cues. Not medically important but it helps explain what is going on overall.

Highlighting the last 2 years:

BBS10 encodes a vertebrate-specific chaperonin-like protein and is a major BBS locus. Paper published in May.

This was a really big deal – BBS10 accounts for 25% of mutations in BBS. It's the same mutation regardless of race. We can now detect the BBS mutations in 80% of BBS patients! Most genes accounted for 5% or 3%. Now that the detection rate can be very high, companies may be interested in developing a test for it.

Finally, DNA diagnostics.

The DNA diagnostic lab at Johns Hopkins has agreed to develop a diagnostic for BBS.

A third option – we have collaborated with a company in Estonia, we have developed an assay that will detect all the known BBS permutations with a success rate of 50 to 55%. It may be an alternative first option that costs hundreds rather than the Johns Hopkins test for \$3000 to \$5000 (don't even know the cost yet).

A diagnostic test:

takes a DNA sample from anybody (carrier, patient, etc.) – are there mutations of the BBS gene in that sample.

For example, you have experience of mis or under diagnosis. How do you make sure you can secure the diagnosis of BBS so you can pursue learning more about BBS. Diagnostic test will do that.

Suppose you have a BBS child who has the mutations. You have a younger child who may be too young for symptoms. A diagnostic test. would tell you if the younger child was affected.

In a year, may have the ability to test *in utero*.

[Another example, not from Nico: Suppose you have a sibling with BBS and you want to marry and have children. It could be a great relief to discover you are not a carrier. Or, if you discover you are a carrier, you could test your fiance and might find out that he is not.]

Has to be affordable, accessible. It isn't yet but it is progressing forward.

MICE

The variability of BBS in humans has been captured in mice with BBS (created in the lab).

We now have mammalian tissue (from mice) to analyze. Many things in mammals are constructed similarly.

Sense of smell

Once we understood about cilia we thought: Cilia of olfactory neurons are probably defective. If we are right about cilia, BBS people must have trouble with their sense of smell. Why has no one said anything to date?

If you never could smell, you don't know you can't.

Showed slide of surface of the nose of a mouse. Mouse needs smell more than eyesight and recognizes social environment by smell. The cilia in a mouse's nose carry receptors.

When you can't get odorant receptors at the surface, you don't have the ability to smell.

When a neuron is stimulated, it sends an electrical current to the brain. We evaluated the ability of BBS people to smell (conducted the test on many at the conference two years ago). Our results showed:

- 50% of BBS patients have normal sense of smell.
- 35% have no sense of smell.
- 10-15% have reduced sense of smell.

How the research progression works:

Worms have cilia for detecting their environment – cilia are also important in smell in a mouse – a mouse with BBS doesn't smell – try it out in humans. We did and now you have the results.

Sense of smell may be included in the primary diagnostic test for BBS – doesn't hurt, costs \$10.

Other profound findings from the mouse:

Mouse embryos – in some kind, the head was open and the brain was growing outside the skull. We looked at older mouse embryos near birth – certain constellation of features caused by a particular situation in genetics that we already understand.

Imagine growing things on a flat surface – need to know your angle and height. If you can't spatially organize yourself like that, the organization is going to go crazy. The best example of this type of architecture is in the ear. The way we hear is because we have these structures that look like a hedge, like columns. They get bent back and forth as sound hits them. The structure of these things is precise – it is V-shaped. If you can't grow the V and it is flat, you will have a hearing impairment. In the BBS6 mouse, the structure is one defective way. In BBS4, the structure is mostly all ok but some V's are not there at all – this means you miss certain sounds, not others. Deafness is not part of a BBS diagnosis but some BBS patients have hearing similar to “aged” hearing, missing ability to hear certain sounds. Auto-acoustic emissions: if you don't have the correct V, the “twang” is affected. Think of it like pulling on a harp string and letting go and it goes “twang”. If the string is defective, the “twang” is different than it should be. So a 20-year-old BBS patient has the hearing of a 40-year old. It is not quality of life impeding, not life-threatening, but it is there.

Zebra Fish

Have been able to create BBS in zebra-fish. We can generate thousands of these embryos in a week. A lot of problems can be studied. Can demonstrate how embryo cells are not behaving properly.

kidney understanding and progress

We now know that the same thing happening in the zebra embryo is happening in the mouse kidney. Kidney cells should be flooded with Beta-catenin. It helps cells decide whether and when to divide or differentiate. The BBS defective cilia – they divide rather than differentiate. That is a big component of many variations – enlargement, dysplasia, and others. Now we have a quantitative biochemical signature!

I have 150,000 chemicals to check, trying to get a certain activity to reduce. If it turns out well, in 10 to 20 years, we can prevent many of the kidney problems.

Screen 150,000 compounds -- Find the pathway -- Cure the fish -- Cure the mice – move to humans.

BBS Mouse Tail immersion assay

How well can mice sense temperature if missing BBS proteins? [A word about terminology: if I say a mouse has BBS4, I mean that in both copies of this recessive gene, he is missing the normal proteins associated with BBS4. To have a BBS gene is to be missing those BBS proteins.]

Need temperature tests.

I cause moderate temperature changes. The mice with BBS, don't care. It takes a much higher temperature before they respond.

There is nothing wrong with the cognitive function of the mouse. It is getting incorrect signals or no signals. The problem appears to be at the innervation of the skin and the interpretation re the environment based on bad data coming from the skin.

Olfaction, hearing, eyesight, (taste?), and touch are affected by cilia.

If you are missing BBS1 proteins, (that is, we say "you have BBS1") there are very few neurons where they are needed in skin. Suppose we measure difference between two locations. People with BBS have a diminished but not lost variability in their ability to sense temperature. Across most moderate temperatures, can't tell the difference between the temperatures at two locations. When one is really hot, they probably CAN tell the difference. They seem to have an extremely high pain tolerance.

We have a formal skin sensation test we want to get volunteers for:

light touch – cotton

pain - sharp stick (not severe, a poke)

vibration – sound a tuning fork; lay it on the skin; see if the person can feel its presence.

proprioception – jt position sense – may have to do with the sensory neurons in the skin

temperature differences

two-point drawing-compass – cannot tell there are two points until they are very far apart – they will say there is only one pt. – because of difficulty sensing the differential between nearby locations.

Difficulty sensing shape and weight.

If you or your child have BBS and you are willing to participate in testing (or to have your child participate in testing), Dr. Katsanis (Nico) wants to conduct the following:

1. Perform a temperature test
2. Perform a skin sensation test (see above)
3. Obtain a small skin sample

Find a (sensory integration) neurologist in your area who is willing to do them. Get contact information and give it to Dr. Katsanis. Dr. Katsanis will contact the neurologist with the test information so that all of them are conducted in the same way. No, Dr. Katsanis cannot reimburse you for what you will be charged. Ask the neurologist about cost ahead of time; explain it is for research; you may get a better deal.