

Disorders of Sex Development – Unexpected clues from two subjects with rare blood disorders - Pediatric Grand Rounds-12-13-24-Meeting Recording

December 13, 2024, 1:31PM

59m 5s

● **Kamat, Deepak M** started transcription



Kamat, Deepak M 0:10

I started recording. Oh OK.

Good, good morning and welcome to pediatric grand rounds.

Just two quick announcements.

Number one is this is the last grand round of the year 2024.

We'll meet again on January 10th, 2025, so we'll have three weeks break for grand Rounds.

The other announcement is this is a reminder for quarterly HealthEquity rounds. This afternoon at 2:30 PM.

The location is changed and is going to be.

LEC 3.102 B in medical school library I have put the address, the place of equity round in the chat box.

You can look at it and please try to attend at 2:30 PM today. So with that is my great pleasure to introduce my friend Doctor.

Vadanapudi Ravindranath, who is a hematologist, oncologist and professor of Pediatrics at Wayne State University School of Medicine.

He received his MBBS degree in India and completed his Pediatric residence and Fellowship in Pediatric Hematology, Oncology, Oncology.

From the Children's Hospital of Michigan, he has practiced pediatric hematology oncology for over six decades.

He has done pioneering clinical studies and developed treatment protocols in pediatric leukemias, both for accurate lymphoblastic leukemia and echid myelogenous leukemia.



Leigh Shapleigh 1:27

Play.



Kamat, Deepak M 1:34

He also has conducted research in the broader range of non malignant blood disorders, particularly in congenital and autoimmune disorders of brain cells. He has published extensively and given presentation at national and international conferences.

On topics in the field of pediatric hematology, oncology today is gonna discuss disorders of sex development.

Unexpected clues from 2 subjects with rare blood disorders.

Doctor Ravi, the floor is yours.



Ravi 2:03

Thank you so much, Deepak.

And I'm honored to give the final grand rounds of the year for your group. I suppose that is across the country, that's.

Probably true, but anyway so it's.

A pleasure to be with you all.

We had a few little technical glitches, but we're ready to roll.

And I apologize for what happened last year and hopefully.

This will go smoothly so.

The you might wonder why.

A hematologist is talking about disorders of sex development.

But where I start now?

I was involved with two patients and I'm going to be talking mostly about these two cases in which there was a very significant hematologic issue and as well.

Significant.

Disorder of sex development. So the.

And here are the these are the two publications that are related to this.

So these two patients and I will start.

Going forward, so first of all, I should say that I don't have any conflicts here.

Then I recommending any treatment and I'm not advocating any, you know, commercial products.



pat herndon 3:35

Play.

R

Ravi 3:37

So the the there's always this question about whether subspecialists practice, holistic medicine or not. And I think you will see that I probably do.

This they and.

In patients with multi system disorders, there is always a clue to what may be going on in other organs than the primary reason you are seeing them.

When you solve the.

Problems in one system.

Typically, they provide clues to what is happening in the other system.

The blood cell abnormalities occur in patients with disorders of sex differentiation, and so those are the things I'm going to touch upon.

Along that line, I came across this obituary on Robert M Solow, who had one Nobel Prize in economics, and.

1997 and last December he passed away.

And there was.

A obituary in the New York Times and his he said that the approach that he used.

In solving problems was to understand a piece of the puzzle as completely as you can.

And he's thinking of that, if you understand a piece of the puzzle completely, it opens up close to all other pieces of the puzzle in that puzzle it's self. It's very appropriate for us to keep this in mind.

In looking at clinical patients and solving the undiagnosed issues in the patients.

So let's talk about sex determination. Sex differentiation first.

But there is this concept called sex determination, which is basically determined by your chromosomal sex.

So if your XY it's male ** is female.

As all of us know.

So in the early.

And this is determinate conception.

And at 8 or 10 weeks of gestation, the gonads, though, have the potential to go either way, either to testis or ovaries.

And subsequent events determine whether you get the male phenotype ultimately or female phenotype. So in.

Some critical aspects of this.

The main differentiation are identified.

On the left side of this diagram, you see that for this.

Xy.

Fetus, they did potential gonad.

The the Y turns on certain hormones and.

Certain genes result there is increased production of anti malarial and hormone AMH which were shut for short.

And once that happens, the if malarial ducks, which did confirm the female eugenital track they get suppressed.

And Wolff and Ducks sort of take over and you get male phenotype. Now if in this process anything goes wrong.

You might end up with.

The loss of Wolff and Ducks and presence of Malarial duck system and you end up with a female.

In our time, you are the fetus of sex.

Still could be chromosomal sex, of course, is unaltered.

Here it's only the phenotypic sexes.

This what we assume to various disorders of sex development.

And there are various classifications out there, and I found this to be a simplified version of it.

So you have more, you can classify them into.

Sex chromosomal disorder anomalies like 45 X.

Or X or like for example Turner syndrome.

And then various combinations of XXXXY chromosome and Klinefelter's, which there are two excess in this male phenotype.

Then you have purely disorders of in the females and I will not really be discussing this.

That much further.

But one important thing that always comes up is the congenital adrenal hyperplasia, where the because of the P-450 acid or reductase deficiency, they don't produce.

I mean they start producing.

Dihydro.

Androstenedione. And because of that, it gives rise to this.

The realization.

Presented both, but then you have a host of 46 XY disorders and.

PH **pat herndon** 9:04

What happened?

R **Ravi** 9:05

You may have heard of the things syndrome called Swears syndrome.

In which because of the mutations in the microbes from and some other genes, you end up with.

Xy sex reversal.

Most important thing is that there is this androgen insensitivity syndrome, which is probably more common of these 46 XY disorders in which the androgen receptor and androgen androgen receptor interaction is disrupted.

So the androgen can be defined as a disorder resulting from complete or partial resistance to the.

Oh, biological action of androgens in an XY individual.

And they typically have normal testes.

So if the gonad is.

Biopsy. Sometimes it may be in gonad or.

But there is age appropriate production of androgens. Yet there is a female phenotype.

And that's because the.

Androgen receptor androgen interaction is interrupted.

It's caused by.

Mutations in the androgen receptor itself.

And this is a.

The this is a schematic of it. Depending the most common mutation is this what's called.

V-582-F it results in complete androgen insensitivity and some other mutations like if there is.

A instead of.

JA **Jerri Abrams** 10:55

And they only come.

Yes, well, it was just the wrapper from the fish. That was kind of stinky, but I double bagged it. Sweetie, I already have a.



Kamat, Deepak M 11:06

Thank you.



Ravi 11:08

Thank you. OK.

So instead of funeral in, if it is a Lucene, it's a less damaging and so you get partial androgen insensitivity.

So it depends on the location of the mutation as well as the specific amino acid change.

So look at this this in the same position, depending on the amino acid mutation either complete insensitivity or partial insensitivity.

So let's go back to our cases and I put in the name J, JD, Jindo. She's now.

She's now about 17.

Or actually 18, the second child born to a non consanguineous Caucasian couple.

A routine gynecologic evaluation on the mother.

Revealed an unsuspected pregnancy estimated by.

Sorry about the typo that estimated to be about 12-10 weeks of gestation and amniocentesis was performed to exclude Down syndrome and karyotypes the fetus to have 46 XY.

However, the ultrasound exam, they could not determine the fetal sex.

She continued this fetal monitoring and was found to have fetal heart drops at 25 weeks of age and.

There was no evidence of blood group incompatibility and a second carrier type also showed 46 XY anyway.

The hemoglobin was 4G at the time, so she received 2 intrauterine transfusions.

The with typo of blood. The child had. The infant was type AB actually, and then electrodes in section was done at 3233 weeks of gestation.

Typically she was female.

To sound sure the normal vagina and the uterus.

What appeared to be ovaries.

Which which were later removed and showed streak gonads on the left.

And on the right, the gonads showed rudimentary 75 tubules and ovarian stroma.

So this finding basically exclude essential.

Androgen insensitivity syndrome as a cause of sex reversal.

Because of the XYCGD in in the complete one hour, this genesis was not could not be determined and no further investigations were done.

The child came to see me primarily for the hematologic problems because she was getting transfusions every four to six weeks.

And I was asked to see and the diagnosis of the hemolytic anemia itself was also undetermined.

At that time. But I saw this bed.

So. So we ended up doing a splenectomy 8, four years of age and presplenectomy the hemoglobin is typically.

Range at 7.4 to 9.3.

The MCV was in the normal range and the blood smears really did not give us any specific clues about.

The type of hemolysis.

And the because of the intrauterine transfusions.

And the infrequent transmissions actually, hemoglobin level was not that very, very revealing.

But one thing that was very obvious on the blood smears was that she had a high nucleated red cell count and platelet counts were also increase and there as well the total count the postprandectomy though.

The clinical the humanitarian picture became somewhat clearer and what I noticed most important was that she had extraordinarily high nucleated red cell count after splenectomy.

Along with a very high red count.

This actually suggests.

Some type of hemoglobinopathy.

This is what you see when you.

Connect to me and I can see me. A major arthritis that'll send me an intermediate.

The nuclear cell count shows up very high. So I thought that this is some type of hemoglobinopathy.

So, three months after splenectomy she.

Fetal hemoglobin of 31%.

With rather high hematocrit.

I'm sorry, MCV.

This doesn't did not fit any known hemoglobinopathy.

At that time.

So here is a graphic picture of what happened. Post traumatic look at the extraordinarily high nuclear deterrent.

Fortunately for me, in my general review, I came across this paper.

In.

And it showed that describe this very uncommon.

What they call this anthropoietic anemia.

Associated with mutation in a transcription factor.

It's a transcription factor.

Alph one. The Hal. More clinical hallmark of this case that the erythrocytes lack.

CD3044 on the surface. Whereas the CD44 was present on the lymphocytes and renders.

And then they showed that the.

Hemoglobin evaluation. That was everyone, a hemoglobin.

It turns out this patient was actually first described about 10 years earlier.

And was studied by a number of people, including Peter Agre, who won Nobel Prize for discovering EPO, four in one.

And they suspected that there is persistence of embryonic.

Message in the case. So fortunately I had a flow lab of my own and I didn't have to get pre authorization. I told my.

Colleagues to your back and go do the.

City 44 on the red cells and he was. I remember that conversation.

He was complaining, he said.

Ravi, you know the sample is 2 weeks old and I don't know. So I told him, just go ahead and do it because it's not going to be ambiguous.

Sure enough, here is the patient and the first column.

Here you can see the bottom there's no CD44 expression.

OK.

Where is the parents?

Had CD44 expression normal expression.

Now, what was amazing was that even the nuclear cells had diminished CD44.

So it was obvious that I was dealing with this rare entity.

Just only two cases had been detected with this disorder after them, and so I thought that, well, what an extraordinary setting.

So we went on to.

Do additional studies and confirm the presence of this invitation in one first thing we

did so this breadthares were quite amazing. I had all these very large.

Red cells.

MCV probably was 130 or more.

This cells, probably sacred cells, the bone marrow nucleated drug cells showed abnormal *****.

And then we did what's called Azure Electric focusing and this was done through Maya or Laboratories and we showed the presence of the embryonic hemoglobin light in the previously published case. We did a bunch of studies.

I won't go into it.

We confirmed the mutation by Sanger sequencing.

And you can see that the imitation.

So here.

At Quran 973 it is G to the protein level and I mean I said this 25 it is a glycine to. I'm sorry to lysine and you can see the.

The the.

And normal.

Grothamic acid in the mother.

And then this is the infant. This is a heterozygous mutation, most likely a homozygous mutation is not tolerated.

So we come to this summary.

So we have identified the mutation and.

The.

We confirmed that we have the third known case of this disorder, but we had several questions that remained.

So why were the why did this child have 46 XY sex reversal? If the chromosome testing had not been done?

Pre Natalie, I don't think clinically.

One would have suspected.

Sex reversal in this case anyway, because she was a phenotypically female.

So she had.

Some other.

Issues. She had a mild hearing deficit, although it looks like that has spontaneously improved.

In the beginning it looked like she might have been had developmental delay, but that's seem to have.

Resulted in she's A is actually going to University of Michigan.

She's a junior there and everything looks like.

It's going normally well, but she has some level of proteinuria which we are still investigating.

So so.

So today there are about 15 known cases of.

For this this KLF one mutation.

And some are females. I'm sorry. Some are phenotypically and.

Chromosomal males. But even the first case that I just described to you there is.

A.

Oh, there's a second.

I'm sorry the second case I so the first one was a 46XX female and she's from Denmark and she apparently has been doing pretty well.

Actually I don't know if she had any children.

The second case is one where the mutation was discovered and.

That child had.

By fate, scrotum and.

Micro penis ensured growth retardation, and that's a 46 XY.

Case and then this is our patient.

That we talked about just now.

And I'll go into a little bit more detail later.

Then there are now at least two other.

Cases in which there is a 46 XY sex or personal.

One of them is recently published. That child is from.

Ukraine and was investigated in Germany. There is an unpublished case in Nebraska that I know of that also 46 XY sex reversal and actually.

The the fact that she was a 46 XY female was discovered when they were investigating the cause of hemorrtric anemia and the whole exome sequencing suggested she is actually XY and.

The identified limitation then we published recently a case from.

Virginia, where the fetus is.

Phenotypic male although there is micro penis and.

In this case, which is apparently quite clinically significant.

So what?

It shows is that this mutation?

Some reason is interfering with the.

Gonadal male gonadal development.

Now in 46 XY.

Fetuses and what is happening?

This is a so so far what has been known about.

PL. Of one is it's actually it has a nickname erythroid ek1 F in the beginning because it is hard to have role only in the erythroids maturation and no other known functions of this.

Transcription factor are known in any other cell system.

So here we have 8 controls, the IT influences the normal maturation of erythroid cells.

Through the various pages.

So it also regulates the fetal hemoglobin to in blue to the development of the adult hemoglobin in the red cells.

And this is untrue.

OK.

Let me so the the the fetal hemoglobin.

Regulation is.

Primarily directed by this gene, called BCL 11, A. When the BCL 11A binds to the beta globin locus, it suppresses gamma and allows for the Gamma Delta beta delta change to synthesize.

So the.

OK, love one regulars. The amount of BCL 11 a that is produced and that way it is regulating the gamma to beta switch.

OK.

Now let's come back to this sex development.

So there is this concept called sex determination, which is determined by the chromosomal sex and then sex differentiation, which is the further development of the phenotype itself of the fetuses going from.

In the early fetus, they this is not determined and but basically the phenotypic sex is.

Determination starts around 8 to 10 weeks of gestation, is complete by first trimester.

And the.

But what?

What is happening here?

Is it's just not enough that you have male like chromosome.

You need various other elements to all work in unison to determine the male phenotype.

The images graphically depicted here, and it appears that the one of the primary determinants.

Of the whether the Bipotential gonad goes into.

Oh oh.

Testicular differentiation versus ovarian differentiation that is determined by the Sox 9.

Transcription factor and Sox 9 is the expression in the cells keeps changing if the Sox 9 is low.

It goes.

Into the ovarian phenotype High Sox 9 pushes it into the testicular phenotype.

Excuse me.

And but there is a complex interplay in this diagram. I like it because it shows you so many other things are influencing other genes are simultaneously overexpressed and underexpressed.

And this.

Complex.

Interplay of many genes eventually determines the the phenotype.

But the main players are really that are determining the.

Ovarian differentiation.

Ovarian survival.

Went for an RSPO one with the beta catinon and the male phenotype is determined by FGF 9 and Sox 9.

Now the the little bit story about FGF 9. So far they've been mutations of Sox 9 and. X chromosome.

I'm sorry, but mutations in SRY gene itself and others that result in this ambiguous genitalia and the partial.

Mail, dys morphology.

So far the there's no known case of FGF 9 mutations or deficiency that results in.

Sex reversal, except FGF 9 knockout mice.

Typically there is 46 XY sex reversal, so that is where the importance of FGF nine has been recognized by this knockout experiments.

Now come back to this summary.

This is my.

Adaptation of some of these things that are going on, so the IT turns out the.

There is a common origin for.

Gonads as well as hematopoietic system. That is what is called the aorta, gonad Mezonephros Ridge and from which both the definitive hematopoiesis as well as the gonadal tissue evolve.

And different genes are regulating these.

Developments. So there's this.

Gene called SF1.

Now actually it's called NR5A1 steroid Genesis factor one, SF1.

And they are required for survival of the.

The gonad itself.

OK. And then once the gonad survives?

And you.

Under the influence of SR5, Sox 9 is.

Expressed and along with FGF nine, it favors development of testis and if you are on the other side.

Data containing went for.

Push the bipart.

So this balance is very critical and if and this is what results in the ultimate phenotype so but the testis itself is not enough.

As we know from the androgen insensitivity syndrome, that if you don't have androgen, androgen, androgen receptor interaction you get.

The androgen insensitivity syndrome and there are several other genes involved or notably.

The.

Secretion of anti malarial hormone.

And secretion of the testis.

Oestrogen itself.

So there is based on the mutations in various genes.

So you get different types of.

This more mismatched for example.

Hhh.

And they're important in labial fold fusion and formation of LS and prostate. So forth.

OK.

So. So the important thing to know that there are many genes that are contributing

to the ultimate phenotype development.

So the the question and these are various syndromes.

You might have heard of Dentist syndrome.

I should only only thing I'm going to say here is that I was fortunate to.

Study under dentist Doctor Dresch himself.

In Pittsburg, during my pediatric or training then you have.

So-called camp for me syndrome. It is due to mutations in Sox 9 and they have a lot of skeletal abnormalities.

Swayer syndrome is a gonadal.

They have strict gonads, the either.

Multiple genes are involved here.

It could be a mutation in.

Sride itself that is causing.

Swear and some other jeans as well, OK.

We talked about androgen insensitivity syndrome.

So my challenge was here.

What has happened to see if I can come up with a hypothesis about what is happening in our patient? So we we have excluded. So what we did is actually we did exome sequencing and she does not have any skeletal abnormality.

She is not otherwise syndromic OK so.

I excluded camper milligrams.

And so forth on that.

The androgen insensitivity syndrome had been already excluded.

And so we needed to do additional work up.

So I went to. This is the hormonal.

Studies that we did hormone studies first thing I did is the anti malarial hormone, and I was puzzled by this extraordinarily high level of anti malarian hormone.

In this child and so the IT looks like that itself was not enough to confirm the suppress the malaria index and confirm the male phenotype.

So, so we did some studies starting with looking at some markers in the exercise gonads.

The these are the gonads and the HI section.

Basically, she had strict gonad on the on one side and there was some philippian. I'm sorry. Some miniferos tubules and ovarians from rudimentary 71st in the in the right ovary.

And we collaborated with the group in Helsinki and did these immunohistochemistry studies assured that the Gator for there and most importantly the?

Antimalarian hormone and the steroidogenic factor 11R5A1 was normally expressed, so we still were left with.

No clear clue of what's going on, and so we.

Decided to do X home sequencing and evaluate it with the help of a bioinformatics person.

And we we looked a number of genes that.

Are important for the survival of bipotential gonad tests and ovary.

And and this was a really a huge task because this child had 32,000 single nucleotide polymorphisms throughout the genome.

So you had to filter out what could be causing the.

Mitigation. So anyway, I want to make this is story short and my colleague.

Not that Craig is a bioinformatisation, and we analyzed each of these snips in.

Of the sex determining genes, it turns out that none of them were individually pathogenic.

But what impressed me in this one is that there was a sniper polymorphism. Virtually every one of the.

Genes involved in the sex determination, including emx, two data for.

Sox 9 but these are all.

Were considered non pathogenic by what's called the insulin core.

Testing there are these algorithms that determine whether this. I mean this nucleotide.

Changes interfering with translation of the gene or transcription itself and so forth.

But none of them rose to the level of.

Being individually pathogenic, so we were still left with.

How difficult?

Not decision to make are resolving this issue, so let's come back to the KLF one KLF one itself is this is was first identified by Jim Baker and this is the recently published a big review and this is the ribbon structure and.

D Delanie 38:29
You go to.

R Ravi 38:35

The and this is a mutated gene. The main thing to remember is that you are going from a glutamic acid in the wild type to a.

Positively charged lysine in the.

The mutated 1, so it could have aberrant non target off target effects besides the editor itself.

So we we did.

We were actually is fortunate.

In this we did whole exome sequencing on the peripheral blood primarily to understand the changes in the erythroid cells.

But when the Craig compared the expression level of all of the genes and determine what's put out, what's called a waterfall plot.

The IT gives some surprising results for us.

Unexpected results.

So.

If the body's on the top about the red bars are increased expression, blue bars are decreased expression.

So easy to follow.

And there's a science here. Are still indicated the process indicate statistically significant difference.

So, not unexpectedly, the KLF one.

Was over expressed.

You can see that anterior anterior hormone was overexpressed.

The SF1 and R5 a one was very highly expressed.

Are other genes the expression levels?

The the green dots below are DST related genes, OK.

What struck me in this when I saw this waterfall plot?

Was that the most significant suppressed gene was FGF 9.

It's completely surprised me.

And then would independently confirm this by targeted expression of FGF 9. By the way, these were, by the way the values that you see.

But actually the relative expression in the patient compared to the average expression in the parents.

OK.

That's what we did here.

So here we show the.

The that the the patient.

The.

The FGF nine was relative decreased relative to the expression in the parents.

OK, this this is our patient.

And this is a second patient from.

OK.

So we confirm that targeted expressions today is also showed decreased expression relative to the expression in the dependence.

So now the question comes up is why is the FGF 9?

Decrease.

Is it from the mutant KLF one doing something or is it there is some other mutation in FGF 9 itself that's causing the?

Decreased expression.

One was that the.

There are a number of polymorphisms in this child, actually 5 polymorphisms.

Are present so individually.

None of these polymorphisms.

They have a certain frequency in the general population, and none of these by itself is pathogenic. OK. However.

The there is the emerging notion that the density of polymorphisms.

I play a role because it works this way.

For example, let us say.

A mutation is resulting in.

5% decrease in the synthesis of that protein.

A 5% decrease by itself is not pathogenic.

But if you have four of them, suddenly it's no longer 5% decrease.

It is 20% level. So this cumulative effect may be relevant in some cases.

That is the concept that is coming out and actually you were shown in.

One Willebrand protein that multiple mutations might actually lower the level of one willogen protein itself and to a clinically significant range. So. So at the moment that's where we are.

But the FDF 9 is.

Probably low in our patient because of all this poomorphisms. Then the question comes up is.

OK, but why do all other?

Why do you have this range of?

DST in the other patients for example, I know of there are probably about 7 males.
Finished K.

It's a big males bond with this disorder.

And two, three, have a complete sex reversal.

Two have a partial sex reversal.

And then they remainder are phenotypically normal.

So why is this variation occurring?

And that's where we are now.

We are.

This is a little bit unsolved, but we have a hypothesis.

And my my notion is that the.

Is CD40.

And it has no.

Seemingly no abnormal effect on the sex development in the females.

So we think what is could be happening is the FGF nine level in conjunction with
some other factor is probably.

Is ineffective in blocking wind for beta catenin and most likely scenario is that the.

Loss of CD44 expression is playing a role because FGF 9 requires a cofactor. In this
case CD44 for binding to and activating the FGF receptor. Fgfr 2 tyrosine finances.

So we know that in the red cells anyway, the four a CD44 is completely.

Unexpressed there a total loss of CD4CD44.

This be happening in the.

Germ cells leading cells in the testes, and that's the reason why you have.

The FGF 9 signaling is completely deceptive.

The only thing I can tell you that the mizanka stem cells that differentiate into
leading gem cells express CD44. So if that is disrupted.

Then maybe this relatively low FGF nine level is of importance.

So we're currently working on this and this is unproven.

Hypothesis at this point and we're continuing to work on this, OK.

So so in my patient, we think it is a combination of decreased FGF 9, decreased CD44
that is contributing to the.

Phenotype. So maybe I'll have to come back at some point and prove.

So this is a summary of what is happening.

The FGF 9.

Is non functional and therefore the the default the. I'm sorry Wolfen.
Did not survive and you have a female and she doesn't have the ovary.
They didn't become going to over his head because the.
The SRY probably.
This this alone is not sufficient.
To come for a full ovarian development, OK.
Now I will switch to in the next few minutes.



Kamat, Deepak M 47:28

Really, we only want 10 minutes left.



Ravi 47:31

Yeah, I'm going to finish that.



Kamat, Deepak M 47:32

OK.



Ravi 47:34

So the next case is a case of.

Rare disease called piccolo, dermal neutropenia, clericuzio type.

This is caused by a mutation.

In a gene called USB one.

The USB 1 gene is a component of the.

U 6.

SM RNA which?

Is.

No.

Accession of the introns and putting the gene together.

But they have a multiple decided the the primary manifestation is a new they have a skin disorder which is the Apache area, patches of erythema and the skin, and that's where the term 5 comes in. One of the things that's now has been known for a long time.



Delanie 48:12

Play.

R

Ravi 48:31

Is that they have leucopenia and neutropenia.

The the arrow is pointing whatever body he has so fine they have increased the sinusopulmonary infections to the extent that sometimes in the very early infancy and some patients have been investigated for CF and CFTR was negative.

They have body changes and they have also neuronal neuropathy, but importantly they have hypergonorrhea.

The mayors exhibit Hyper Gonorrithis.

And the question comes up is why is this USB1 mutation causing hyperinical?

So I had.

I was involved with two brothers with this disorder, so we started investigating actually.

The the family in detail.

Because one of the siblings.

Had.

A.

A very had a wound in the perianal area.

Would not heal, so I was asked to see the patient and see if giving.

GCSF will spur the healing, but what we when we did, we gave GCSF and even though it increased the white count, the wound healing it had no effect on the wound healing.

And I gave systemic an intralesional GM-CSF to promote monocyte.

Plasticity.

And push them towards the wall healing monocyte.

And that also did not.

Result in own healing and the boy actually.

Still has a tiny wound after four years and is slowly healing.

So here's a mutation in this child in the in this family, this is sibling is wild type.

Normally you have.

Ag in this location and you can see the patient instead of G it is.

A.

And then and then the here was the father is a carrier and has both EG and A and its.

Oh, but what we noticed was that the monocytes were very, very abnormal looking in this family. They showed these virtuals and they have a low monocyte count, low

neutrophil count, and then you have this maculated, monocytes. And so we investigated the monocyte plasticity in this family. And what?

I show here.

Is that the we use this panel car using CD14 and 16?

And normal.

Controls you have about.

510% of the.

The monocytes express.

CD16. So you see clearly 16 positive monocytes, whereas in the patients there was a virtual absence of CD16 positive monocytes. We were the first ones to show this in this group of patients.

Expression on the the.

On the NK cells and neutrophils was intact.

So it looks like it's a specific problem in monocytes monocyte.

Fail to evolve or respond.

And and do not express this CD16 marker.

So we did a. So the, the, the voice had hyper.

Micro penis and.

Actually, there was a very disturbing part of that evaluation and we did a number of hormone studies and we find that the.

Virtually they have this hyper gonadotropic hypogonadism. OK, End Time 1 levels were very high.

So it's actually the.

The question is unresolved.

So we were doing these experiments on the monocyte plasticity.

And we found that basically the monocytes were stuck in the pro inflammatory configuration, I think because of the time I will just.

Complete so.

And not only that way, what we found was that the the cyclic amp levels.

We suspected a problem in the cycle KMP signaling because several.

Cyclic amp pathway genes were decreased in expression in the siblings relative to the control and this was statistically significant. What our algorithms are they use now.

The question we measured the cyclic amp levels.

In the plasma and found that in the patients acyclic amp levels were decreased.

So the question comes up is what is its relevance to the whole clinical picture now

this?

It turns out that the so one of the things that cyclic ampiculin does is it activates multiple targets in primarily the chloride channels, and that will CFTR relationship. Might be important, but it also.

Oh, activates the androgen.

Are gonadotropin signaling because the gonadotropin?

Receptors are G couple receptors and the G couple receptors are susceptible to cyclic AMP signalling.

So on on binding of cyclic AMP to the regulatory units, the.

The IT releases the protein.

Kinda C protein kinda. Just put in kinda as a ncpk and PKC end results and activation of the genes.

But depending on the signaling that starts there. So.

So here is a graphic picture of it. G proteins upon by so the.

Cyclic.

Amp is sequestered normally through these genes, called a caps and others, and once the recyclic amp signalling occurs.

These. I'm sorry. Protein kindness sequestered. And they are released once the cyclic amp binds to the is activated and that activation is terminated by phosphodiesterases.

So the in the absence of.

Proper cyclic amp signaling.

It's possible that in our patients you have decreased gonadotropin signaling, so I'll close out with this thing.

It's not as simple as Rishi sunak.

The ex Prime Minister of U, KS.

A man is a man and a woman is a woman. That is just common sense, but I think it's sex. Determination is highly complex.

And you can get unexpected clues for unsolved.

Disorders of sex development.

By looking at the total picture and you might get clues from.

Actually, blood cell disorders and I gave you illustration of two. Think I will stop and then take questions. Thank you.



Thank you, doctor.

Thank you, doctor Ravi.

Let's see if anybody has any questions.

So this second patient, did he have a lot of infections with monocyte?

 **Ravi** 56:47

No, the the primarily the infections were.

Early on in childhood, they had Sinop pulmonary infections and actually they have abnormal sweat chlorids. So the chloride, the the they are very high chloride levels.

 **Kamat, Deepak M** 56:59

Oh, I see.

 **Ravi** 57:04

They thought it was CF, but the CF gene on multiple exome sequencing studies.

The C.

FTR, gene itself was normal.

And there's no pathogenic mutation.

 **Kamat, Deepak M** 57:18

OK.

 **Ravi** 57:18

The other infections that seem to be mostly.

Well, actually were recently was admitted with another.

Pulmonary infection. They seem to have problem with infections in the lung.

 **Kamat, Deepak M** 57:34

Yeah.

 **Ravi** 57:34

They don't have sepsis.

They're not.

None of them died of sepsis, but wound healing seems to be a primary problem in these patients.



Kamat, Deepak M 57:38

OK.



Ravi 57:43

Someone patient required some amputation and others were treated with hyperbaric oxygen and so forth.



Kamat, Deepak M 57:52

Anybody has any other questions?

I don't see any, just a quick reminders that this was a last brand round of year 2024.

We'll see you again on January 10th, 2025 and also reminder for the quarterly HealthEquity rounds today at 2:30 PM.

In LEC 3.102 B is in the chat box, you will see it.

See the.

The room location of the room in the chat box.

Thank you all.

Have a wonderful holiday season and we'll see you next year. Thank you.



Ravi 58:43

Thank you, Deepak, for inviting me and thank you all.



Kamat, Deepak M 58:43

You all part in the.

Thank you.

Bye bye.



Ravi 58:47

Great holidays.



Kamat, Deepak M stopped transcription