

# Mock – JC. Script

Presenter: JC presentation.

## 1.Gioele

Ok if there are no immediate questions,  
let's take it from the first figure  
and see if there are points of criticism.  
In Figure 1 we see a staining they performed on a coronal section  
from human fetuses... this is a very precious sample obviously.  
What do you think about this staining?

[ PAUSE OF REFLECTION]

## 2.Luca

At first, I took the figure a bit for granted...  
But now that I am looking at the "1B" staining, I wonder  
Is the antibody they used for detecting the human HTT  
working for both normal HTT and mutated HTT?

## 3.Alessandro

This is a very good point.  
But I think they specify in the paper that the antibody  
they used has been proved specific for both normal and mHTT.

## 4.Gioele

Ah you are right, and after all, the HTT localization claimed is pretty evident  
Are there other points on this figure?

...

so let's go to the Figure 2.

Do you have anything to bring up about this figure?

[SILENCE]

Maybe I can start...

Looking at the co-immunoprecipitation experiment,  
I see something strange, did you see it as well?

## 5.Morgane

Do you mean the strange correlation between the different components of the junctional complex, right? (Gioele: "Yes!")

Can I explain it? (Gioele: "Go on!")

There is an incongruence. Between figure 2E and figure 1E.

Figure 2E, the Co-IP, shows a connection between HTT and the other proteins, including PAR3.

Now, if you go to Figures 1E and F, you see all the proteins claimed to be connected, having a good correlation with HTT but PAR3 has actually an opposite trend.

How do you think it is possible?

My only explanation is that maybe they are not interacting directly...

(Alessandro raises his hand)

(Gioele: "I have an idea, but I see Alessandro wants to make a point, go ahead")

## 6.Alessandro

Well, I think this is a very good point.

If we look at the staining we see a partial correlation with HTT and maybe when HTT is mutated, only part of the complex is disrupted.

If that is true it might change the interpretation of part of the paper...

## 7.Luca

there are other papers they cite in the discussion

that show the connection between PAR3 and HTT.

So the data is not very clear on this point but

they don't claim something new and they cite the right references.

## 8.Gioele

Ok, more data on PAR3 and HTT interaction would be nice

but Luca, you are right,

it is not crucial for them to be able to make their claim.

Does anyone have other questions about Figure 2?

## 9.Morgane

I noticed that in Figure 2C there is something strange about the GFP expression between control and disease.

Do you see the same?  
I mean, there shouldn't be any qualitative difference  
of in the GFP between the two conditions

**10. Alessandro**

Well, it is true...  
It looks like in the mutant, the GFP gets stuck in the vesicles  
or anyway, it is differently localized compared to the control.

**11. Gioele**

Yes, it is true, super nice observation!  
In a peer review, I would ask the authors  
But now we can only speculate.... right?  
We have limited time, we must focus on the key evidence and claims.  
I say we go to the next figure!

In Figure 3, we have experiments about the cell cycle,  
Do you think we should discuss something?  
Maybe, Luca, you could start?

**12. Luca**

In Figure 3E they show data on the single-cell tracking.  
They measure the velocity in the G1 migration and G1/S transition.  
but plotting very few points...  
In G2 migration, there are a lot of them!

What do you think is the reason for that?  
Also, are the few points for G1 enough to sustain the difference  
in the cell cycle, they are making a claim on?

**13. Morgane**

Well, actually, it can be due to technical reasons,  
In this experiment, they are growing 250um-thick coronal sections  
of the mouse brain *in vitro* to perform the single-cell recording

If some radial glial cells move inside the tissue beyond what the  
microscope can penetrate you will lose the cell  
And you have to discard those cells from your experiment, right?

#### **14.Alessandro**

Well in this case you will have the same problem for the G2 migration instead, there are plenty of points there.

I think it is due to technical reasons and to the experimental planning...

Were they desperately trying to make the G2 migration turn out significant?

(Gioele: That would be data manipulation!)

#### **15.Gioele**

Ok, I guess we will never know,

The p-value at the end of the day was small enough to make the statement about the cell cycle statistically relevant.

But maybe there is something suspicious technically here if the claim they make here is shaky maybe they could reinforce it with an orthogonal technology maybe EdU/BrDU double staining. Let's agree on that maybe...

So, let's move to the next part.

Do you want to highlight something here?

#### **16.Morgane**

They have a final section where they claim that

"mutant HTT biases neurogenesis towards the neuronal lineage"

For that claim, they consider primary cilium disassembly as an important proxy

The measurements are shown in Figure 4C...

they measure the length of primary cilia

the result is a significant difference but the effect size is relatively small.

In this situation, technical aspects matter...

One point is that the sections are 14um.

Maybe 14um is too short to span the entire length of the cilia, no?

They could have used specific markers for the extremity of the cilia or 3D reconstruction to make sure their measurements were correct

#### **17.Alessandro**

Well, but if they quantify in the same way for case and control

Does it matter if the length is not complete in absolute terms?

What matters is the comparison, no?

I agree, but there is something that may be more worrisome...  
The length of primary cilia can depend on the cell cycle!  
In the G2 phase, the cilia are longer  
in the other phases it gets disassembled and it is shorter.  
Considering that the mutant condition has more radial glia in the G2 phase  
it can be that the cilia are generally longer because of just that.

**19.Alessandro**

Actually, it makes sense with the fact that  
I think that they cannot claim it is longer without  
looking for colocalization with cell cycle markers.  
They need to do that experiment!

**20.Gioele**

Ok this is a very nice observation, we might have nailed a key criticism.  
Now what do you think about the last claim?

They claim that HT disease leads to fewer PAX6-positive progenitors  
and more TBR2 positive ones compared to controls.  
This means that HT disease patients have fewer cycling progenitors  
and they tend to differentiate earlier.

This is a very important claim. What do you think?

**21.Alessandro**

I noticed they don't show the staining for the mutant condition in the figure.  
Maybe they show it in the supplementary...  
but I think that considering how important it is  
they should show it in the main figure.

**22.Morgane**

Well, actually, there are not even in the supplementary!  
and also claim the same for the mouse model they use...  
and again, in the supplementary, they show only the control condition!

### **23. Gioele**

Ok, this seems really data-hiding!  
I don't understand how they passed the review process.  
I am happy we touched another critical point!

Does the other group want to ask for any clarification or make any comment?

[DEAL WITH QUESTIONS]

So we arrived almost at the end, and now let's have some room  
for more general points discussion.  
Shall we discuss the significance and future directions?

### **24. Luca**

I was thinking  
Do you think a similar developmental impact may be possible  
for the determinants of other neurodegenerative conditions?  
For example, Alzheimer, Parkinson or other poly-glutamine diseases  
such as spinocerebellar ataxia type 1 in the cerebellum?

(Gioele: that would be cool)

### **26. Morgane**

I was thinking  
Here, they use human donors with around 40 CAG repeats.  
Maybe studying different CAG repeat lengths  
could lead to a better understanding of this neurodevelopmental alteration.  
Imagine if it could explain why some patients develop the pathology  
at a young age and others in old age!

## FINAL Gioele

Okay so now we are at the end.

To conclude, what we just discussed:

- The association between HTT and PAR3 in the apical junctional complex there it would be nice if experiments could be performed to dissect their interaction but overall are outside the main scope of the paper.
- The observation is that mutated HTT gives strange GFP localization at the apical side of the electroporated radial glia cells.  
This was suspicious but we cannot know what it means.
- Then a bit of a statistical point  
The cell cycle difference between mutant and control could be performed again looking at more radial glia.
- The experiment to assess the length of the primary cilia in radial glial cells is not satisfactory.  
Other experiments have to be performed to analyze why mHTT leads to apparently longer primary cilia.
- For the last claim of the author, they are hiding the data.  
What they show is definitely not enough to say that the mutant HTT interferes with apical radial glia specification.

Overall it the study highlights an exciting finding,  
Methodologically it is not fundamentally bugged but does not really shine  
but some pieces of evidence do not fully appear fully transparent.