



May 2019

Unraveling genetic interactions at the primary cilium

Bailey McCurdy
Western Washinton University

Follow this and additional works at: <https://cedar.wvu.edu/scholwk>



Part of the [Higher Education Commons](#)

McCurdy, Bailey, "Unraveling genetic interactions at the primary cilium" (2019). *Scholars Week*. 84.
https://cedar.wvu.edu/scholwk/2019/2019_poster_presentations/84

This Event is brought to you for free and open access by the Conferences and Events at Western CEDAR. It has been accepted for inclusion in Scholars Week by an authorized administrator of Western CEDAR. For more information, please contact westerncedar@wvu.edu.

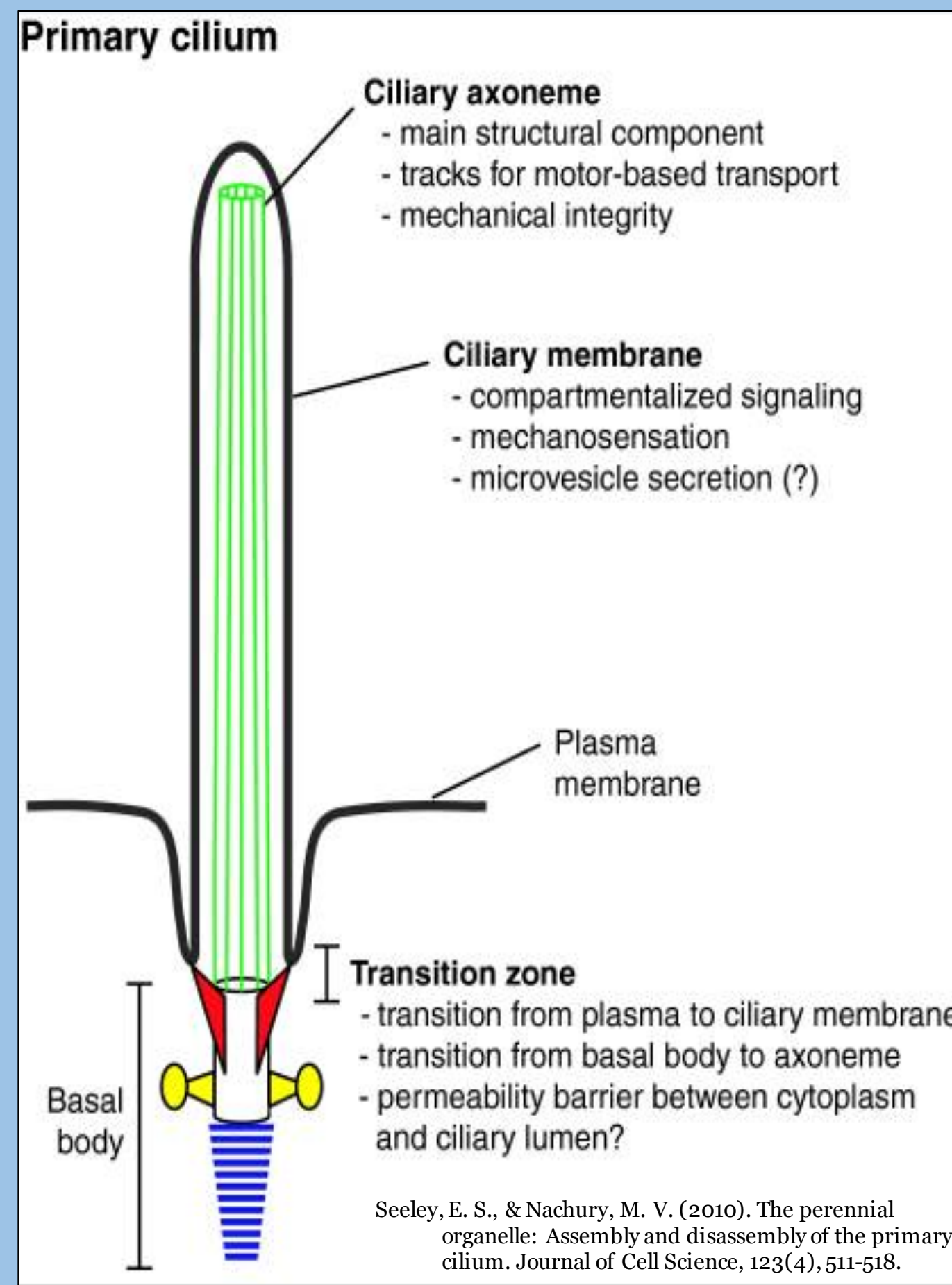
Unraveling genetic interactions at the primary cilium

Bailey McCurdy and Nick Galati*

*Dept. of Biology, Western Washington University, Bellingham, WA

Abstract

Down syndrome is one of the most common genetic conditions in the world, with a prevalence of 1 in 700. Down syndrome is caused by an additional copy of chromosome 21. Although all individuals with Down Syndrome have an extra copy of chromosome 21, the clinical outcome of Down Syndrome varies. Why is this? One possibility is that disruption of other genes that are not on chromosome 21 also contributes to the clinical outcome. I have identified a strong genetic candidate called NPHP1 that disrupts a structure called the primary cilium—a vital signaling structure that is essential for human development. Since Down syndrome also causes cilia defects, this raises the possibility that disruption of the gene NPHP1 makes cilia defects more severe in individuals with Down syndrome. To test if NPHP1 disruption combines with an additional copy of chromosome 21 to alter primary cilium function, I will examine how NPHP1 expression alters cilia in control cells and Down Syndrome cells. I hypothesize that mutations in NPHP1, combined with an extra copy of chromosome 21, increase cilia defects in cells from Down syndrome individuals. If NPHP1 mislocalizes in Down Syndrome cells as compared to control cells, then NPHP1 varies the clinical outcome of Down Syndrome.



Plan of attack

The first aim was to identify a candidate gene that may modify the cilia phenotype in Down syndrome cells. To complete this aim, I used a combination of bioinformatics and literature searching, to identify candidate cilia genes that may combine with an extra copy of chromosome 21 to increase cilia defects. This approach led to the identification of the gene NPHP1 (Fig. 1). The second aim is to test whether NPHP1 modifies the cilia phenotype in Down syndrome cells using the cilia assay described in Figures 2 & 3. To complete this aim I will first completely reduce expression of NPHP1 to observe presence of a modified cilia phenotype. Then, I will reduce expression of NPHP1 to 50% to observe a mild cilia phenotype as compared to the completely reduced NPHP1 phenotype. Next, I will increase PCNT expression to observe a modified cilia phenotype. Lastly, I will examine if an increase of PCNT AND a reduction of NPHP1 expression cause a stronger modification in cilia phenotype than the phenotypes caused by reduced NPHP1 or increased PCNT alone. If a stronger modification in phenotype is noted, I will examine NPHP1 localization when NPHP1 expression is reduced and PCNT expression is increased to determine why or how localization changes and creates a stronger modification in cilia phenotype.

Introduction to primary cilia

The primary cilium is required for individual cells to develop into multi-cellular organisms (Satir and Christensen, 2007). Defects in the function and formation of the primary cilium often result in problems with development (Satir and Christensen, 2007). Primary cilia are important for development because primary cilia function as cellular antennae that receive information from other cells (Gerdes et al, 2009). Therefore, when ciliary signaling is disrupted proper developmental patterning does not occur within the individual (Reiter & Leroux, 2017).

Primary cilia dysfunction in Down syndrome

Down syndrome is caused by an extra copy of chromosome 21 (Hattori et al, 2000). An extra copy of chromosome 21 causes defects in a cellular structure called the primary cilium (Galati et al, 2018). It has been shown that over expression of a ciliary protein found on chromosome 21, pericentrin (PCNT), disrupts the formation of primary cilia (Galati et al, 2018). Individuals with Down syndrome have a wide range of clinical outcomes that differentially impact the development of the heart, the brain and the skeletal system (Ramachandran et al, 2014). I am interested in understanding the cellular origin of these developmental differences and whether they relate to primary cilia. One possible explanation for why an extra copy of chromosome 21 causes a variety of clinical outcomes is that the ultimate disease outcome depends upon other genetic mutations in the individual. This concept is known as the genetic modifier hypothesis and it has been proposed for Down syndrome (Li et al, 2012). To identify candidate genes that could modify Down syndrome outcomes, I have been focusing on finding genes that, when mutated, could combine with an extra copy of chromosome 21 to cause more severe cilia defects.

* "OMIM is a comprehensive, authoritative compendium of human genes and genetic phenotypes that is freely available and updated daily."
 ** "ClinVar aggregates information about genomic variation and its relationship to human health."
 -NCBI, US National Library of Medicine

Cilia assay for quantifying number and length of cilia

To quantify cilia, I first find the number of mouse 3T3 fibroblast cells with cilia. On only those cells with cilia, I quantify cilia length. For example, in Figure 2 there are 10 cells pictured. Of those 10 cells, 7 have cilia (yellow boxes). Figure 3 demonstrates the ability to quantify actual cilia length in micrometers. Fig. 3a shows no cilia, Fig. 3b shows a short cilium at 1.309 um, and Fig. 3c shows a long cilium at 2.964 um.

A. Finding a candidate gene (Figure 1)

Find proteins that are localized to the primary cilium or centrosomes using the centrosome and cilia database (CCDB)

Of these proteins, find which ones interact with pericentrin (PCNT) by reviewing literature.

Filter out the proteins from chromosome 21.

Using OMIM* and ClinVar**, narrow down proteins that are pathogenic when mutated.

Find which of these proteins that when mutated also worsen the clinical outcome of Down syndrome

B.

1555 genes in CCDB

97 genes interact with PCNT

93 genes pathogenic in OMIM or ClinVar

Mutation is correlated with congenital heart defects in individuals with Down syndrome: RAB21, WDR53, NPHP1

NPHP1

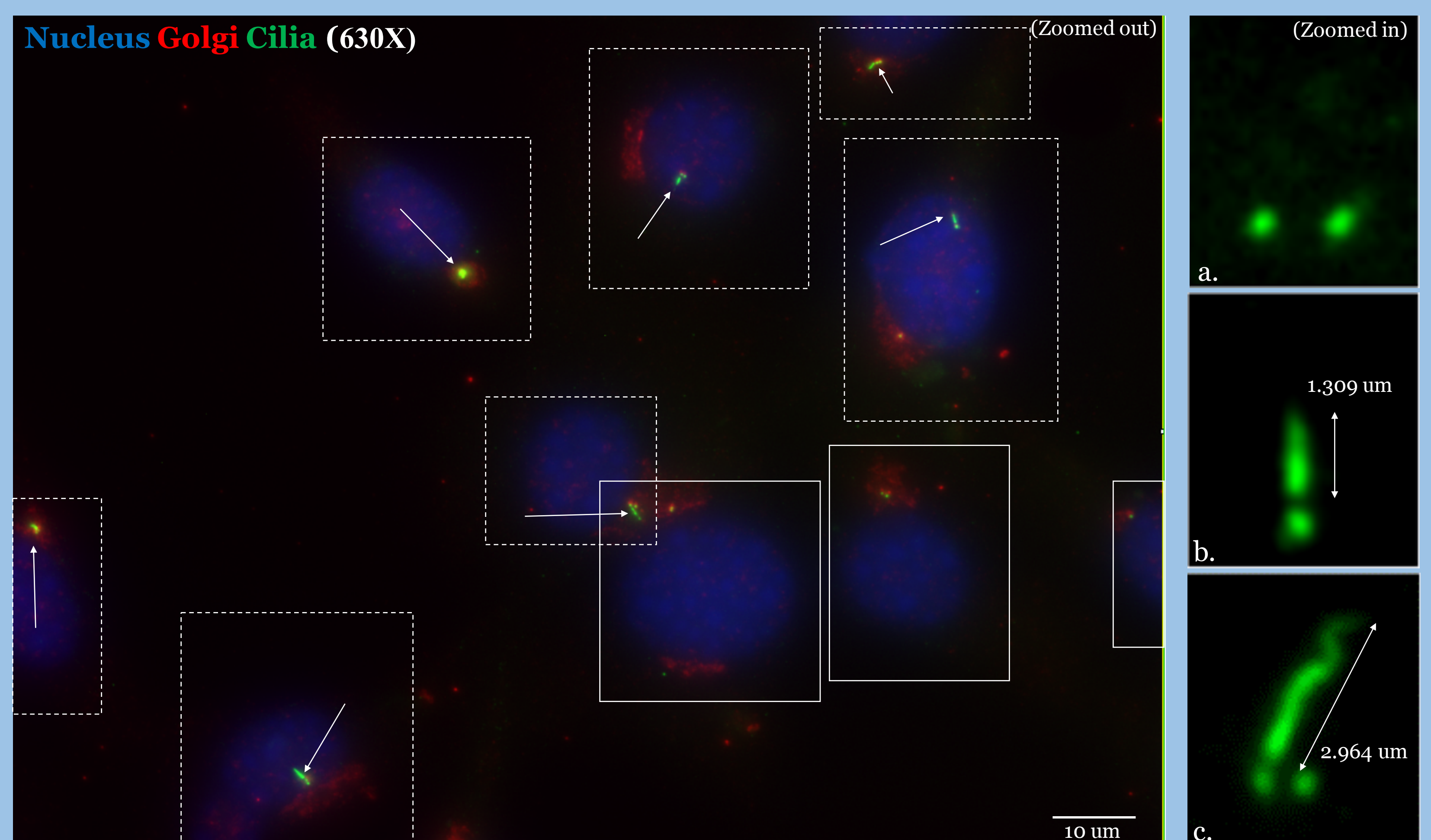
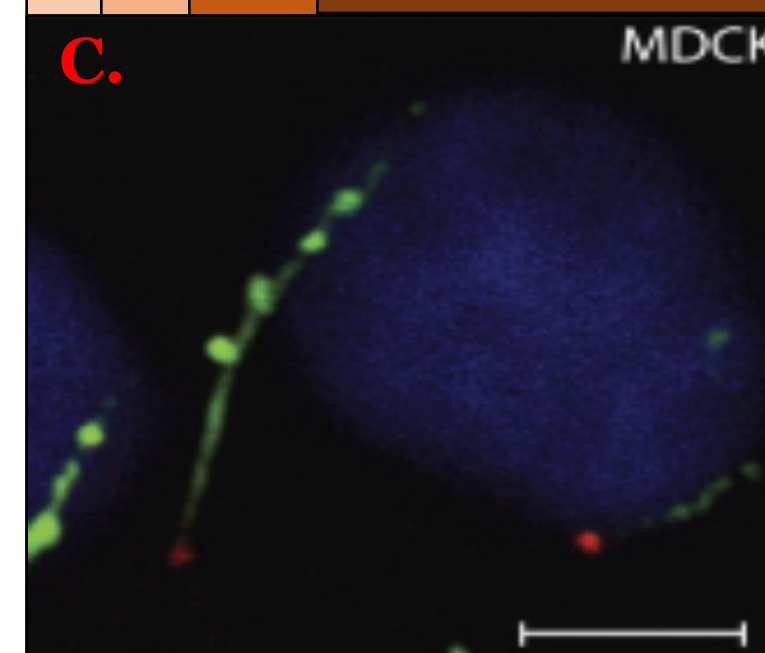


Figure 2

Figure 3

Works Cited

- Galati, D. F., Sullivan, K. D., Pham, A. T., Espinosa, J. M., & Pearson, C. G. (2018). Trisomy 21 Represses Cilia Formation and Function. *Developmental Cell*, 46(5).
- Gerdes, J. M., Davis, E. E., & Katsanis, N. (2009). The Vertebrate Primary Cilium in Development, Homeostasis, and Disease. *Cell*, 137(1), 32-45.
- Hattori, M., Fujiyama, A., Taylor, T. D., Watanabe, H., Yada, T., Park, H. S., (2000). The DNA sequence of human chromosome 21. *Nature*, 405(1), 311-319.
- Li, H., Cherry, S., Klindinst, D., DeLeon, V., Redig, J., Reshey, B., . . . Reeves, R. H. (2012). Genetic Modifiers Predisposing to Congenital Heart Disease in the Sensitized Down Syndrome Population. *Circulation: Cardiovascular Genetics*, 5(3), 301-308
- Ramachandran, D., Mulle, J. G., Locke, A. E., Bean, L. J., Rosser, T. C., Bose, P., . . . Zwick, M. E. (2014). Contribution of copy-number variation to Down syndrome-associated atrioventricular septal defects. *Genetics in Medicine*, 17(7), 554-560.
- Reiter, J. F., & Leroux, M. R. (2017). Genes and molecular pathways underpinning ciliopathies. *Nature Reviews Molecular Cell Biology*, 18(9), 533-547
- Satir, P., & Christensen, S. T. (2007). Overview of Structure and Function of Mammalian Cilia. *Annual Review of Physiology*, 69(1), 377-400.