“Dysregulation of cotranscriptional alternative splicing underlies CHARGE syndrome”
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AUTHORS AND THEIR CONNECTION TO THE CHARGE SYNDROME FOUNDATION:

Catherine Bélanger (PhD candidate) and Félix-Antoine Bérubé-Simard (postdoctoral fellow) (pictured with Nicolas Pilon, second row, second picture) are in the lab of Nicolas Pilon at the University of Quebec in Montreal, Quebec. Numerous others from the lab also contributed to the project.

Nicolas Pilon, PhD (second row, second picture), is head of the Molecular Genetics of Development lab at the University of Quebec in Montreal, Quebec, where he concentrates on research on neurocristopathies (including CHARGE syndrome) using mouse models. This work was funded in part by a grant from the CHARGE Syndrome Foundation.

Seema Lalani, MD, (second row, far right) is a medical geneticist at Baylor College of Medicine in Houston, Texas. She has been involved with the Foundation since the Houston conference in 1999 where she and colleagues drew blood on families in search of genes for CHARGE syndrome (CS).

Donna Martin (second row, third picture), Stephanie Bielas (in purple gloves collecting a sample), Amanda Moccia (second row, first picture), and Anshika Srivastava (not pictured), are in the Department of Human Genetics at the University of Michigan. The lab has been doing CS research for many years, including research on cells from saliva samples collected at conferences. Donna is the head of the Scientific Advisory Board of the CHARGE Syndrome Foundation and a recipient of a Star in
CHARGE award. She has coordinated professional meetings on CHD7 at the University of Michigan in non-conference years.

Summary by Meg Hefner

SUMMARY OF THE PAPER:

Abstract: CHARGE syndrome (CS) is a severe developmental disorder with wide phenotypic variability – every affected individual has a unique set of features. The main known cause of CS is a mutation in CHD7 gene. “CHD” stands for “chromodomain helicase DNA-binding protein” and is a series of genes (CHD7 is the seventh one to be recognized) known to code for proteins involved in remodeling (or altering) chromatin (DNA, RNA and protein that makes up chromosomes) to effectively turn other genes on and off. The causes of CHD7 mutation-negative cases (children with CS who do not have CHD7 mutations) are unknown, at least in part because the pathogenic mechanisms underlying CS remain poorly defined – exactly how does the variant CHD7 protein affect embryologic development to result in the features seen in CS. Here, we report the characterization of a mouse model. These mice have the same features as CS mice but they do not have mutations in the mouse-equivalent of CHD7. These mice were created by insertional mutagenesis of the gene Fam172a (family with sequence similarity 172, member A). We show that Fam172a plays a key role in the regulation of cotranscriptional alternative splicing, notably by interacting with Ago2 (Argonaute-2) and Chd7. Validation studies in a human cohort allow us to propose that dysregulation of cotranscriptional alternative splicing is a unifying pathogenic mechanism for both CHD7 mutation-positive and CHD7 mutation-negative cases. We also present evidence that such splicing defects can be corrected in vitro by acute rapamycin treatment.

Additional summary: CHD7 is the only gene known to cause CHARGE Syndrome (CS). However, the diagnosis remains clinical and many individuals with a clinical diagnosis do not test positive for CHD7 changes. The authors describe a complex series of experiments and interpretation of findings that may help explain some of that apparent paradox.

Many of the features seen in CS are a result of problems in embryonic development of the neural crest. CHD7 is a “chromatin remodeler”, which affects the level of expression of many of the genes known to be involved in neural crest cell (NCC) development. CHD7 may also affect alternative splicing. This paper looks at another gene (Fam172a) that may be involved in some cases of CS and compares mouse and human cells to see how they react in specific situation. As they put it, “we report here the generation and detailed characterization of a mouse model for CHD7-negative CHARGE syndrome.”

Note from Meg: Oops – this “lay summary” is already getting pretty dense. This paper is hard core research. To understand even a summary, it may help to have a short refresher on genetics:

Contrary to what was believed in past decades, each gene does not simply code for one protein; there are too few genes to account for the number of proteins in the body. If you remember your high school biology, you know that, simplified, the process goes from DNA (gene) being transcribed to RNA, which is then translated into amino acids, which form proteins. It turns out there are even more steps in this process. Genes need to be turned on (different genes at different times and in different cells). Chromatin remodeling allows access to the DNA on the chromosomes. Genes themselves are composed of introns (non-coding regions) and exons...
(coding regions). Introns in the DNA must be spliced out so only exons are transcribed into RNA. RNA triplets are eventually translated into a string of amino acids, which then fold intricately into proteins. Sometimes even exons are spliced out (or a different starting point is used), resulting in multiple different proteins resulting from the same gene. This is called “alternative splicing.” CHD7 has long been known to be a chromatin remodeler. Researchers are now finding it may have other roles as well, including perhaps in alternative splicing. Some of these additional roles may be (at least part of) the explanation for why CS is so variable and how you might end up with clinical CS without a mutation in CHD7. A note on nomenclature: genes are always italicized; when it is the human gene, it is in upper case (CHD7), when it is an animal gene only the first initial is upper case (Chd7 or Fam172a).

Toupee is a mouse model for CS but it is not caused by Chd7. As in humans, the features seen in the Toupee mice (see Figure 1) are variable, but the authors went to great lengths to show that the features are analogous to those seen in CS. Once that was established, they looked very carefully at the behavior of NCCs during embryology. This is important not only because it confirms that Toupee is a CS model and helps us understand the origins of the features, but also because if abnormal migration and behavior of NCCs can be treated in mice, that might be important for people eventually. Toupee, it turns out, has a change within the Fam172a gene. So now they had a gene other than Chd7 that appears to cause CS in mice. What does this gene do? The labs did extensive research and modeling to try to determine that. If you are a molecular geneticist, you may understand the complex descriptions, figures and the 30 page appendix that detail what they did. Bottom line: “Fam172a appears to be required for stabilizing protein–protein interactions at the chromatin–spliceosome interface” – in other words, without Fam172a, alternative splicing abnormalities occur.

The University of Quebec team then searched through the non-CHD7 human cells obtained from the Baylor CS cohort (contributed by Drs. Lalani and Campeau), and were able to identify a mother-child pair (both with CS) with a FAM172A variant. Examination of non-CHD7 cells from the Michigan cohort then allowed the identification of a second mother-child pair with a FAM172A variant. [A shout-out here to those of you who participated in research at Baylor and/or the University of Michigan; those are the cells they used! - Meg] They back-checked those variants (intentionally created the same variants in mouse cells) and confirmed that they disrupted the same alternative-splicing functioning. “All these results thus support the idea that impairment of cotranscriptional alternative splicing is a unifying pathogenic mechanism for all cases of CHARGE syndrome.”

Might this finding lead to a potential therapy? Oversimplifying, rapamycin is a chemical which promotes splicing. In cells from CS individuals, splicing events were corrected! They then injected small doses of rapamycin into pregnant Toupee mice, which resulted in a decrease in the incidence of coloboma by 50%. In large doses, rapamycin causes birth defects in mice. Even in the small doses used, it caused apparent growth retardation of the mice.

The Discussion section summarizes the findings and speculates more about the mechanisms involved in the features of CS and comparing that to what is known about many syndromes with overlapping features. Although a lot is known about DNA, transcription, embryology and related topics, there is clearly a lot more to learn. They end with a series of questions and then acknowledge that both Toupee and Chd7 mouse lines will be useful in further investigations.
WHAT DOES THIS MEAN TO FAMILY/PERSON WITH CHARGE?

Nothing directly. This is pure, hard laboratory research. The CS community is fortunate in a way that CHD7 has turned out to be a very interesting gene. That means that despite continuing reductions in NIH funding, many researchers are interested in learning more about it. Studying CS will not only help us understand CS, it will further the understanding of molecular genetics and embryology. In the long term, studies such as this may point the way to therapies that help reduce the burden of CS.

SHOULD I READ IT? SHOULD ONE OF MY DOCTORS READ IT?

The PNAS journal is subscription only, so you may need to pay to access the full article. It is also a research article written for other research professionals. Lots of jargon and technical details, with a 30 page appendix. However, if you are interested and able to get to it, be sure to not only read what you can, but also check out the links to the “Movie” and “Appendix” to see the CS-like features in the mice, a video of a mouse going in circles (vestibular abnormalities), and videos of migration of neural crest cells in embryos. Probably the main thing you will get from this paper is that it is all incredibly complex but we are making progress understanding how all of these things work. This work is not relevant to clinical care at this time – your doctors likely would not be interested.

FULL CITATION: Dysregulation of cotranscriptional alternative splicing underlies CHARGE syndrome

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