Small Mutation, Big Consequences

Targeted mutation in the *stub1* gene causes structural changes of purkinje cells in the zebrafish cerebellum; a novel animal model of human neurodegenerative disease.

Johanna Letzner, Eirik Austad, Elsa Denker, Ståle Ellingsen

The Disease

Name*[1]*:	**SCAR-16 (**autosomal recessive spinocerebellar ataxia 16)
Prevalence*[2]*:	<1:1 000 000
Symptoms*[1]*:	**Degradation** of cells in cerebellum (see figure 1) which leads to cognitive and motoric impairment
Cause*[3]*:	**mutation** in the ***stub1*** gene, causing malfunction in the multifunctional protein **CHIP (proteasome degradation system: protein folding and degradation)**

The Zebrafish

The zebrafish is a widely used **model organism**:

- Short generation time
- Transparent eggs
- Rapid embryonic development
- High number of offspring[4]
- **84% of disease-causing human genes have a counterpart in zebrafish[5]**

Targeted mutations in zebrafish are generated using **CRISPR/Cas9** methodology. Slides of brains from homozygous mutants were stained using **Immunohistochemistry (IHC)** (figure 4) to visualize the structural changes.

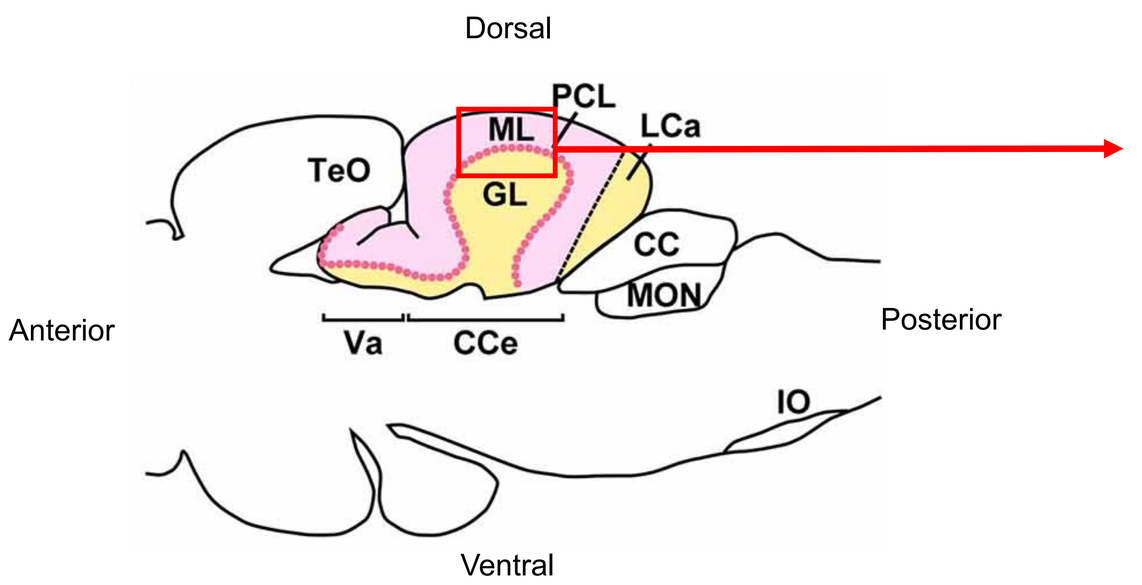


Figure 1: Sagittal view of zebrafish cerebellum showing a schematic illustration of the zebrafish brain. The layers of interest are the PCL and the ML, marked in dark pink and light pink, respectively.

[Illustration]. Available from: https://www.frontiersin.org/articles/10.3389/fncir.2019.00030/full (found: 04.11.2019)

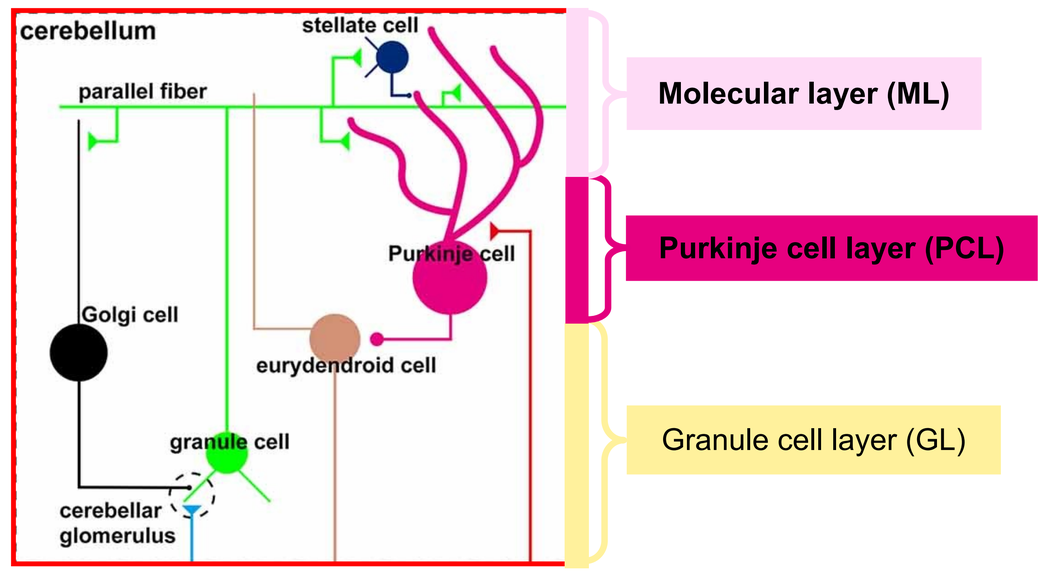
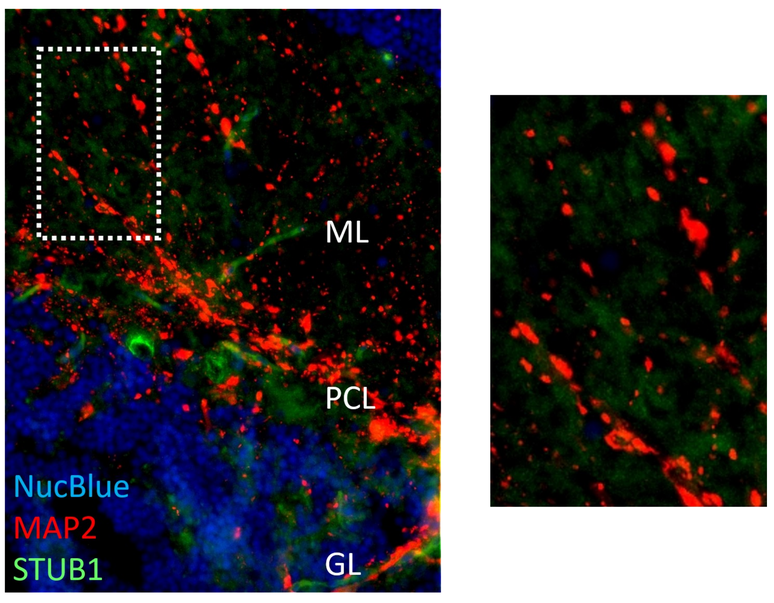


Figure 2: Illustration of the cell layers in the zebrafish cerebellum (see figure 1). Cells of interest (purkinje cells in the PCL and ML) are shown in dark pink.

[Illustration]. Available from: https://www.frontiersin.org/articles/10.3389/fncir.2019.00030/full (found: 04.11.2019)

A: Wild type



B: Homozygous mutant

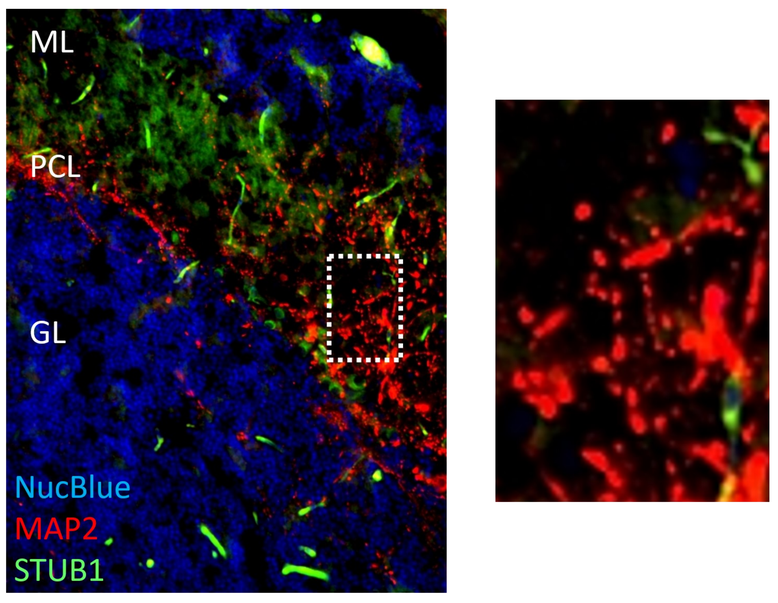


Figure 3: Microscopy images showing the sagital view of the cerebellum (area marked red in figure 1). Figure 3A shows the dendrites in oragnized lines. 3B shows no such pattern and the dendrites have no apparent organization. To obtain these pictures, fluorescent microscopy was used. See figure 4 for a description of the antibodies that were used and the structures they bind to. Marked in A and B are the three cell layers (see figure 1 & 2) and the antibody colors. See figure 2 for information on orientation of purkinje cells and dendrites in the PCL and ML.

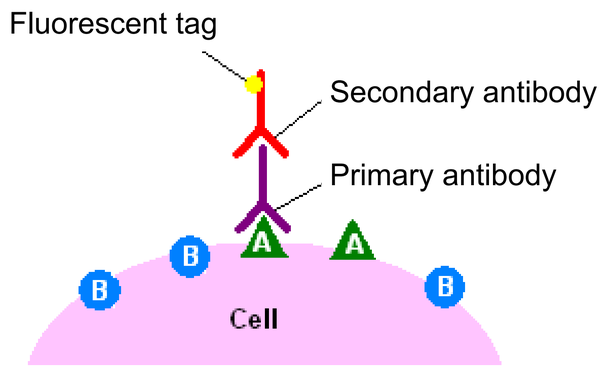


Figure 4: Simplified figure of immunohistochemistry (IHC).

(https://upload.wikimedia.org/wikipedia/commons/3/37/Immunohistochemicalstaining2.PNG)

Antibodies

- NucBlue stains DNA
- **MAP2 stains microtubules in dendrites of purkinje cells**
- Anti-CHIP stains the STUB1 gene

Change in Dendrite Structure

There are clear **lines of marked dendrite areas** extending with their dendrites from the PCL into the ML in figure 3A, and a few cell bodies can be seen in the PCL (green circles). If one were to stain the cellbodies of the purkinje cells in addition (which can be done with an antibody called Parvalbumin) the lines and their origins would be even clearer. Such a pattern can not be seen as easily in 3B. This is because of the structural changes in the dendrites that come from this mutation, which also have been shown in previous experiments in the lab (Parvalbumin IHC, data not shown).

Exactly **how the mutation causes these changes** is still unclear.

What we know is that the targeted mutation leads to **decreased proteasome activity** of CHIP[1]. Proteins marked for degradation might thereby accumulate, and it can be speculated that this is part of the reason why the dendrites are less organized in the mutants than in the wild type. **To prove this, extended research is needed**.

References:

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3) *Shi. C.* et al. (2018) Disrupted structure and aberrant function of CHIP mediates the loss of motor and cognitive function in preclinical models of SCAR16. *PLOS Genetics.* 14(09). DOI: 10.1371/journal.pgen.1007664
4) Tyler M., Kozlowski R. (2018) DevBio Laboratory: vade mecum3. *An Interactive Guide to developmental Biology*. Sunderland: Sinauer Associates Inc. Chapter 15. (read: 02.11.2019)
5) Howe, K., *et al.* (2013) The Zebrafish Reference Genome Sequence and its Relationship to the Human Genome. *Nature* 496(7446). DOI: 10.1038/nature12111

University of Bergen
Department of Biological Sciences (BIO)