

Prenatal study of the Gli family proteins in the cerebellar development under *weaver* condition



SERGI ALACID ROMERO, Bachelor's Degree in Genetics
Facultat de Biociències, Universitat Autònoma de Barcelona

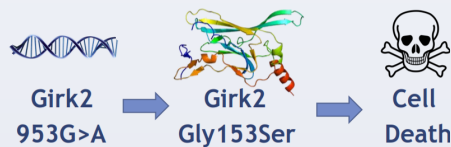


INTRODUCTION

Mice carrying the *weaver* (*wv*) mutation suffer from a wide range of symptoms, and all of them result from the death of several cell types in the Central Nervous System (CNS). How the *wv* allele affects the development and so the phenotype isn't yet clear. One of the most relevant pathways during cerebellum development is Sonic Hedgehog (*Shh*), which regulates gene expression through the Gli transcription factor family. These proteins are deeply involved in granule cell (GC) proliferation, and since their depletion is a major feature of the *weaver* condition, the study of the prenatal expression of the Gli family could shed some light upon the mechanisms that link the *weaver* genotype to its phenotype.



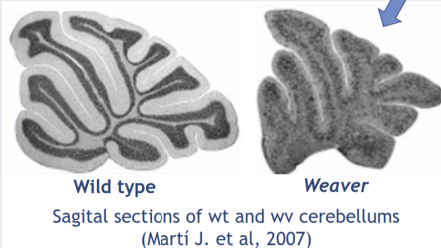
The *weaver* mutation and condition¹



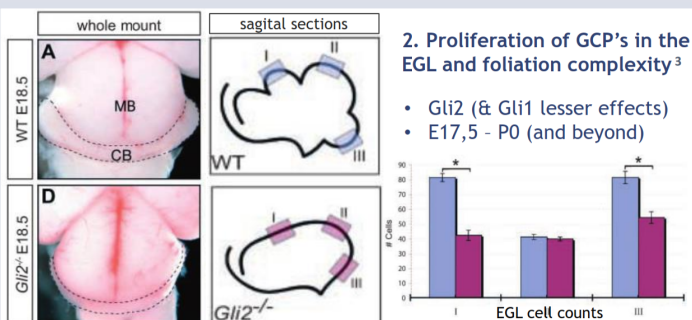
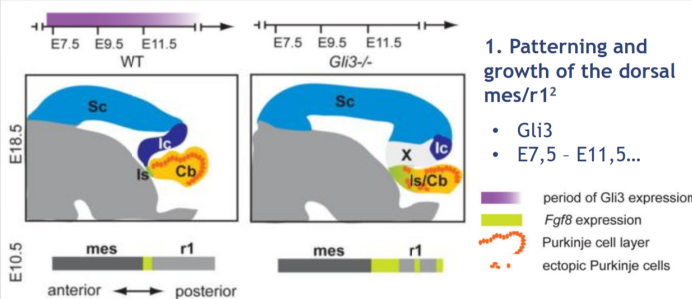
Cerebellar abnormalities:

Structural deficiencies

Depletion of GCs, PCs and deep cerebellar nuclei neurons



Key steps in the prenatal cerebellar development mediated by Glis



OBJECTIVES/WORKING HYPOTHESIS

The main objective of this study is to check whether the expression of the Gli proteins in *weaver* mice is correct or not.

More specific goals would be to inquire into the specific effect of each Gli member to the *weaver* phenotype, by searching expression disturbances at specific stages:

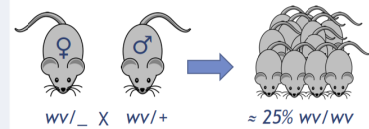
- *Gli1* and *Gli2* during GCP proliferation in the EGL (E17,5 - P0...)
- *Gli3* during the patterning and growth of the dorsal *mes/r1* (E7,5 - E11,5)

The working hypothesis of this study claims that Gli transcription factor proteins are indeed affected by the *weaver* condition during embryonic development and thus, they contribute to the resulting phenotype.

METHODOLOGY

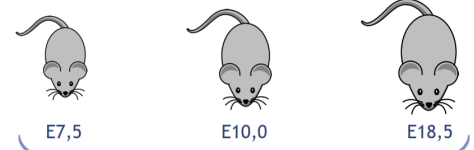
Mutant stock maintenance

First heterozygous mutant parents from Jackson Laboratories ME, USA

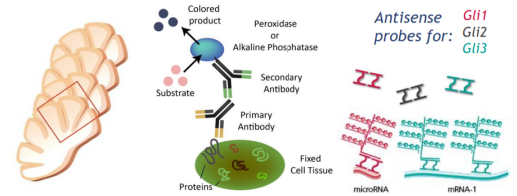


Genotyping DNA from tail tissue allows us to characterize and select desired mice embryos⁴

Selected *wv/wv* and *+/+* mice grow until desired stage of development

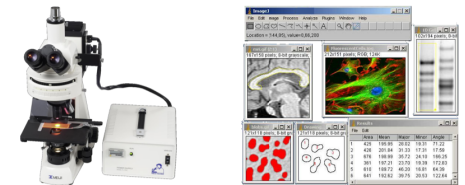


Histology, immunohistochemistry and RNA in-situ hybridization⁵



Fluorescent microscopy and analysis

Quantitative & Semiquantitative *wv/wv* vs *+/+*



EXPECTED RESULTS/DISCUSSION

We expect to confirm our working hypothesis and find significant differences between the expression of *Gli1*, *Gli2* and/or *Gli3* from *weaver* to wild-type mice, at least at some stage of the cerebellar development.

If that's the case, much speculation on how *Girk2* affects Glis could be made, based on an hypothetically impaired *Shh* signaling from PCs to GCPs:

- Constitutively activated *Girk2* changes PC's efferent target microenvironment.
- Constitutively activated *Girk2* interferes with the expression of GDNF and/or with the appearance of their receptors in PCs

It is worth noting that poor research has been done in the context of prenatal life in *weaver* mice, and thus new discoveries could be done in that field.

Furthermore, since *weaver* serves as an animal model for the cerebellar ataxia, findings in their embryonic life development could help to early diagnose - and treat - the hereditary cases.

REFERENCES

- ¹ Martí, J. et al., Purkinje cell age-distribution in fissures and in foliar crowns: a comparative study in the *weaver* cerebellum. *Brain struct. Funct.* 212, 347-57 (2007)
- ² Blaess, S. *Gli3* coordinates three-dimensional patterning and growth of the tectum and cerebellum by integrating *Shh* and *Fgf8* signaling Sandra. *Changes* 29, 997-1003 (2012).
- ³ Corrales, J. D., Rocco, G. L., Blaess, S., Guo, Q. & Joyner, A. L. Spatial pattern of sonic hedgehog signaling through Gli genes during cerebellum development. *Development* 131, 5581-5590 (2004).
- ⁴ Cavalcanti-Kwiatkowska, R., Raisman-Vozari, R., Ginestet, L. & Del Bel, E. Altered expression of neuronal nitric oxide synthase in *weaver* mutant mice. *Brain Res.* 1326, 40-50 (2010)
- ⁵ Corrales, J. D., Rocco, G. L., Blaess, S., Guo, Q. & Joyner, A. L. Spatial pattern of sonic hedgehog signaling through Gli genes during cerebellum development. *Development* 131, 5581-5590 (2004).