# Oral presentation

# Molecular mechanisms underlying neuroepithelial/ependymal denudation in the hydrocephalic *hyh* mutant: spatial and temporal expression of alpha-SNAP and N-cadherin

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## Background

The hyh mutant mouse develops fetal-onset neuroepithelial/ependymal denudation that precedes cerebral aqueduct obliteration and hydrocephalus [1,2].А hypomorphic point mutation (M105I) in alpha-SNAP protein has been identified as responsible of the hyh phenotype [3]. Alpha-SNAP is widely distributed in all mammalian tissues and cell types [4]. It is a key component of the SNARE machinery for membrane fusion and participates at different levels of vesicular traffic of proteins, including transport to plasma membrane [5]. But, why does a mutation in such an ubiquitous protein lead to selective developmental disorders of the central nervous system? How is alpha-SNAP mutation involved in neuroepithelial/ependymal denudation? Considering that (i) the pattern of ependymal denudation matches that of ependymal differentiation [1], and (ii) the ependyma of circumventricular organs, endowed with a special set of junctions, never detach; it is proposed that alpha-SNAP mutation could result in a failure in the adhesion/junction proteins physiology during brain development leading to neuroepithelial/ependymal denudation. The aim of the present investigation was two fold: (a) to study the temporal and spatial expression of alpha-SNAP, NSF, and some proteins involved in intercellular junctions, and (b) to evaluate the importance of these proteins on ependymal physiology and stability.

## **Materials and methods**

(i) Brain samples of non-hydrocephalic (wild type) and hydrocephalic (mutant) mice from the hyh strain (B6C3Fe-*a*/*a*-*hyh*) were studied by immunocytochemistry (IMC) and transmission electron microscopy (TEM) at various developmental stages. Protein homogenates from telencephalum, mesencephalum/brain stem and cerebellum were analyzed by Western blot. The expression levels of mRNA encoding for alpha-SNAP and NSF were analyzed by semi-quantitative PCR. (ii) Ependymal explants obtained from adult bovine Sylvius aqueduct were cultured for 24 hours and used to evaluate the role of adherens junctions and N-cadherin in ependymal stability. Basically, after validation of this ex-vivo model, N-cadherin functional blocking assays in 1DIV explants using specific antibodies and competitive peptides were performed. The effect of N-cadherin blockage was evaluated by light microscopy (quantitative analysis), IMC, and TEM.

#### Results

(1) alpha-SNAP and NSF are preferentially expressed in the CNS and at early developmental stages; (2) alpha-SNAP is preferentially expressed at ventricular lining; (3) in mutant animals, the decrease of alpha-SNAP protein varies at different stages and at different brain regions; (4) hyh mutant mice present an increase in NSF protein,

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probably due to its overexpression; (5) ependymal cells express N-cadherin but not E-cadherin; (6) different ependymal subpopulations showed a differential expression of alpha-SNAP and N-cadherin; (5) functional blocking of N-cadherin led to (i) changes in N-cadherin immunocytochemical pattern, (ii) ultrastructural modifications of adherens junctions, (iii) increase of the intercellular space, and (iv) detachment of the ependyma leading to large denuded areas of the explants.

### Conclusion

The selective expression of alpha-SNAP in the brain, and its differential expression at distinct brain regions and cell types may contribute to the understanding of the molecular mechanisms underlying hyh phenotype. N-cadherindependent adherens junctions play a key role in ependymal stability. An alteration in the physiology (traffic?) of N-cadherin appears to be the one of the mechanisms operating in the ependymal denudation of hyh mice.

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#### References

- Jiménez AJ, Tomé M, Páez P, Wagner C, Rodríguez S, Fernández-Llebrez P, Rodríguez EM, Pérez-Fígares JM: A programmed ependymal denudation occurring during the embryonic life precedes the development of congenital hydrocephalus in the mutant mouse hyh. J Neuropathol Exp Neurol 2001, 60:1105-1119.C.
- Wagner, Bátiz LF, Rodríguez S, Jiménez AJ, Páez P, Tomé M, Pérez-Fígares JM, Rodríguez EM: Cellular mechanisms involved in the stenosis and obliteration of the cerebral aqueduct of hyh mutant mice developing congenital hydrocephalus. J Neuropathol Exp Neurol 2003, 62:1019-1040.
- Chae TH, Kim S, Marz KE, Hanson PI, Walsh CA: The hyh mutation uncovers roles for alpha Snap in apical protein localization and control of neural cell fate. Nat Genet 2004, 36:264-270.
- 4. Clary DO, Griff IC, Rothman JE: **SNAPs, a family of NSF attach**ment proteins involved in intracellular membrane fusion in animals and yeast. *Cell* 1990, **61:**709-721.
- Stenbeck G: Soluble NSF-attachment proteins. Int J Biochem Cell Biol 1998, 30:573-577.

