“Lithium as a positive modulator of defective WNT pathway in Cornelia de Lange Syndrome models»

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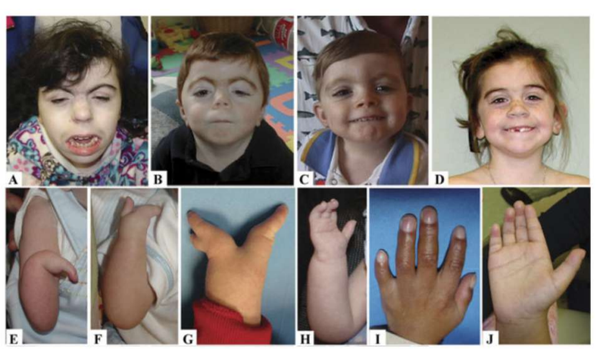
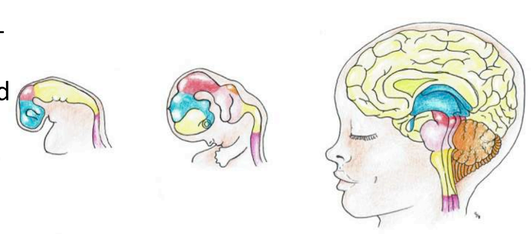
Introduction and aim of study



Dorsett and Krantz, 2009

The cohesin complex is a multimeric system, highly conserved in the course of cellular evolution from the most primitive life forms to human cells. Cohesins are essential Structural Maintenance of Chromosomes (SMC), protein-containing complexes that interact with chromatin and modulate chromatin organization and gene expression. Genetic variants that cause structural and/or functional alterations induce an array of congenital pathologies named "cohesinopathies". It is believed that such malformations arise from deregulation of pivotal developmental molecular pathways. Canonical WNT pathway has been shown to be perturbed in association with central nervous system malformation in Cornelia de Lange Syndrome (CdLS), one of the most characterized cohesinopathy.

In this study, we validated the relevance of canonical WNT pathway and assess the effect of LiCl-dependent activation of WNT pathway in three CdLS experimental models: Lymphoblastoid cell lines from patients, murine Neural Stem Cells (NSCs) and *Drosophila melanogaster*.



Avagliano et al, 2017

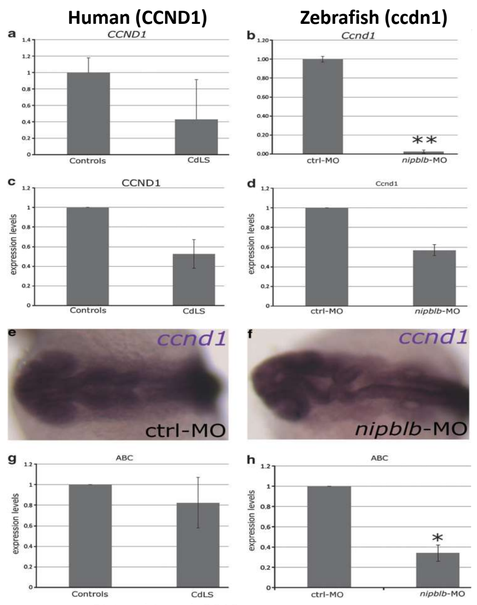
Background

Zebrafish (*Danio rerio*) and patients’ fibroblasts

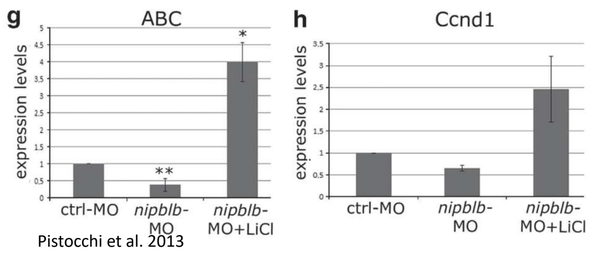
Previous studies on Zebrafish *nipblb*-MO-injected embryos show that wnt pathway expression is severely altered.

The canonical Wnt pathway is downregulated in animal and human models of CdLS. Human (CCND1) and zebrafish (ccnd1) real-time and Western blot analysis in patients-specific fibroblasts and in *nipblb*-MO-injected embryos show a reduction trend of the transcripts in both models.

We can appreciate the reduction of *CyclinD1* (CCND1 and ccnd1) respectively in *NIPBL*-mutated patients and in *nipblb*-loss-of-function embryos compared to healthy controls.

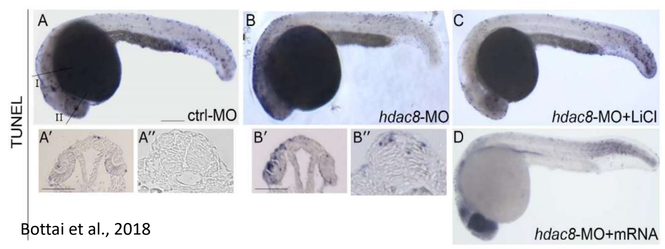


Pistocchi et al. 2013



Pistocchi et al. 2013

Chemical induction by lithium chloride of the canonical wnt pathway can rescue nipblb deficiency phenotype.



Bottai et al., 2018

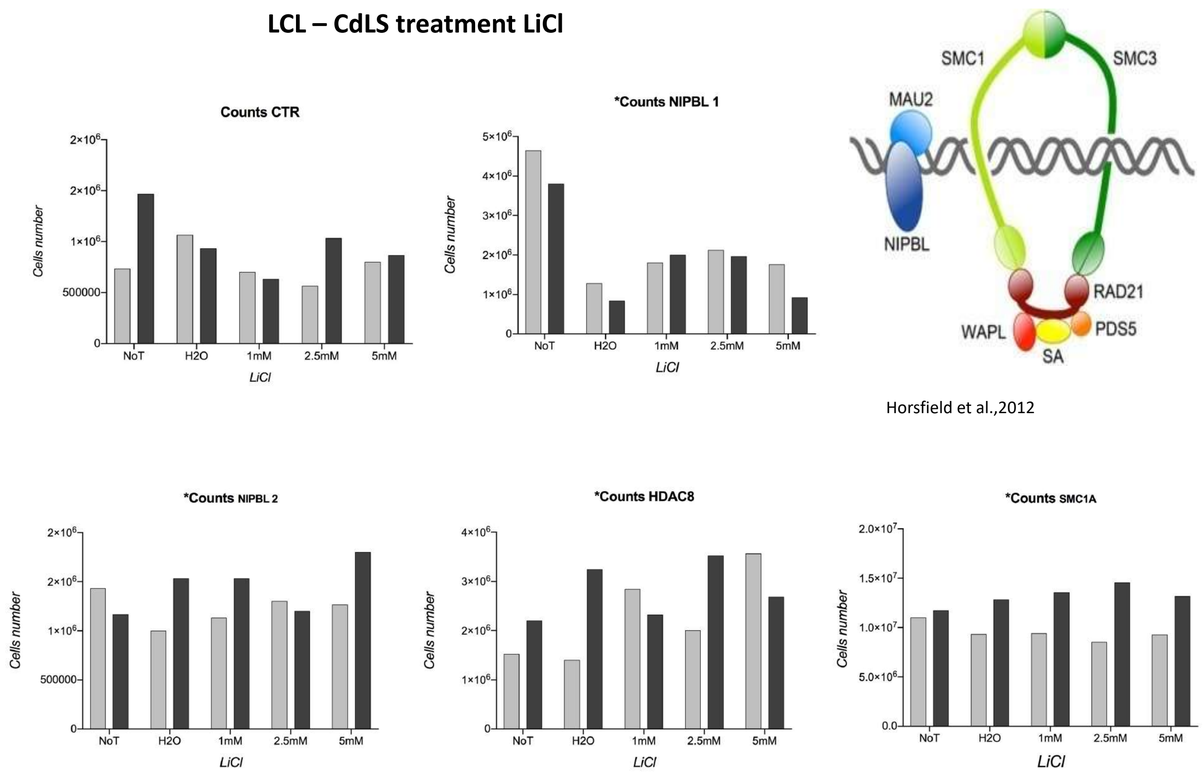
hdac8 loss-of-function zebrafish show Central Nervous System (CNS) malformations and an increase of apoptosis at the level of the midbrain, hindbrain optic vesicles and spinal cord in embryos .

Following treatment with LiCl, TUNEL assay showed significantly reduced levels of apoptosis compared to control embryos .

LCL-CdLS

Lymphoblastoid cells (immortalized lines from CdLS patients) of patients carrying mutations of *NIPBL*, *HDAC8* or *SMC1A* genes and healthy donors were used in these studies. These cells were treated with LiCl 1mM, 2,5mM and 5mM, and proliferation rate were measured.

LCL – CdLS treatment LiCl



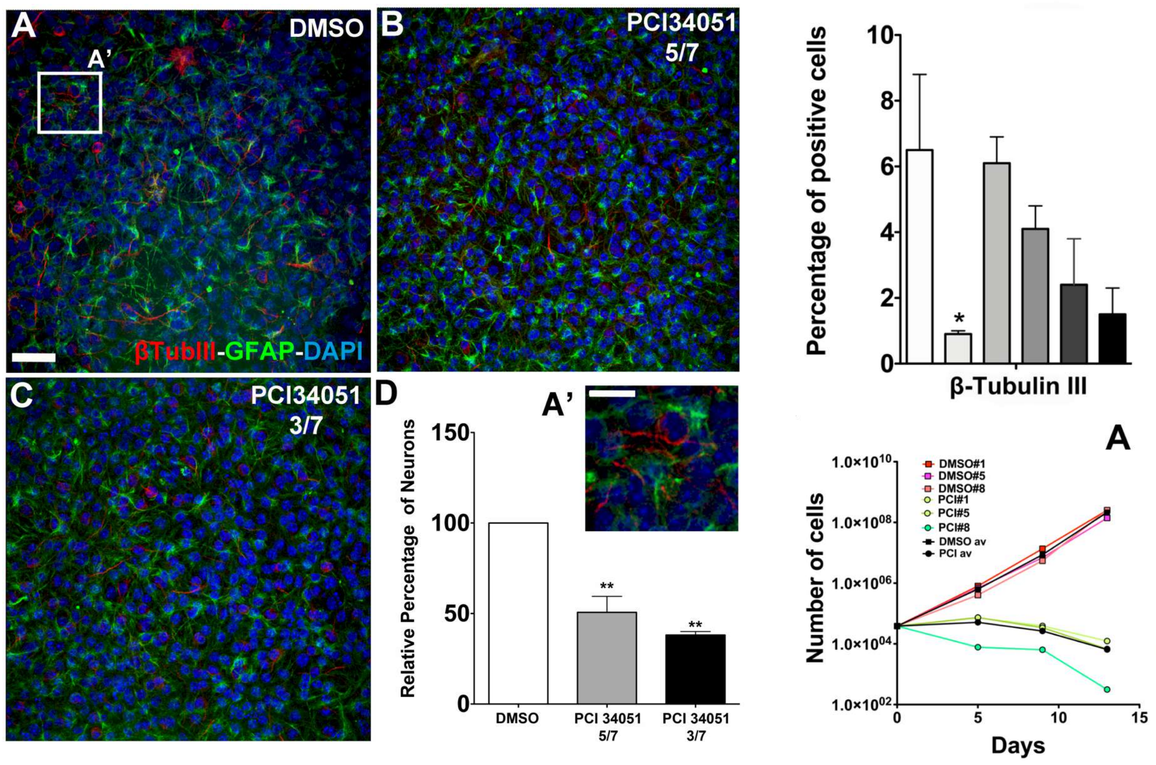
Horsfield et al.,2012

Unpublished data

Preliminary data on lymphoblastoid cells showed no effects on cell death rate in healthy donor following LiCl treatment. And, although with a patient-specific response, LiCl appeared to induce an increase in proliferation, especially in cell lines that were slow-growing compared to controls.

murine NSCs

Proliferation and differentiation capabilities were also assessed in CdLS mouse NSCs.



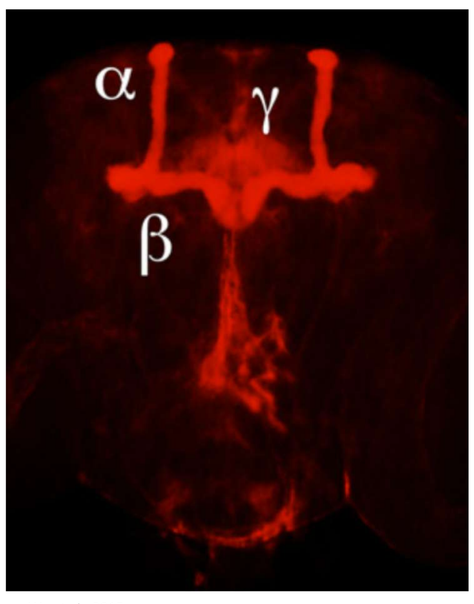
Bottai et al., 2018

NSCs showed reduced NSCs proliferation rate and differentiating capabilities especially towards neuronal lineage.

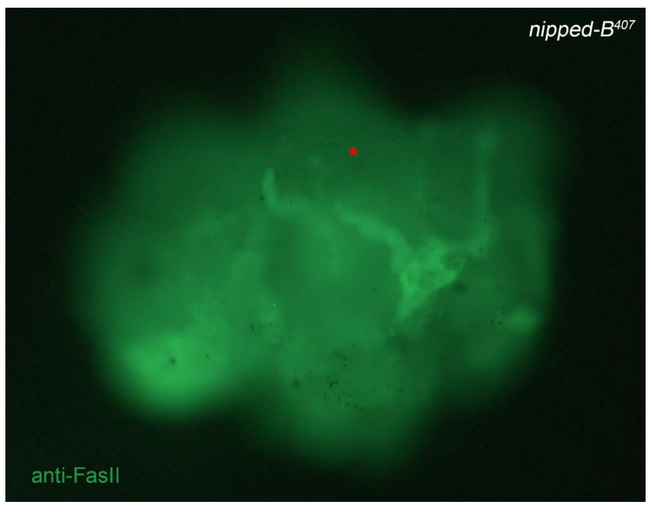
Drosophila melanogaster

nipped-B in *D. melanogaster* is the ortholog of the human *NIPBL* gene and we are testing the mutated loss-of-function allele *nipped-B*^{407}.

Flies were grown upon food added with a different concentration of LiCl. *Drosophila* brains were analyzed for morphological evaluation.



Wu et al., 2015



Unpublished data

Drosophila mutants for *nipped-B* gene display malformations in mushroom bodies (MB), a structure involved in olfactory learning and memory. Treating subsequent generation of flies with 100mM of LiCl, MB morphology was restored in the offspring. These data confirm the pivotal role of canonical WNT pathway in regulating CNS development in CdLS models and pave the way for developing therapeutic strategies.

Conclusions

All these data further confirm the hypothesis that in CdLS is present an impairment of WNT pathway that could, in part, explain the typical neurodevelopmental alterations of this syndrome.
Moreover, these studies could pave the way for future therapeutic strategies.

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