Modulating the WNT pathway in *Drosophila* models of Cornelia de Lange Syndrome

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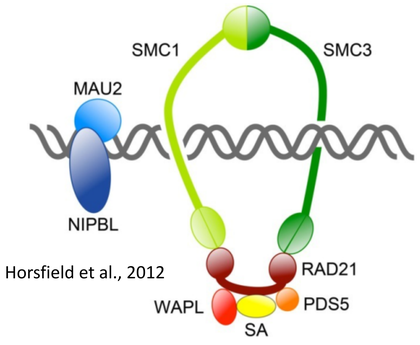
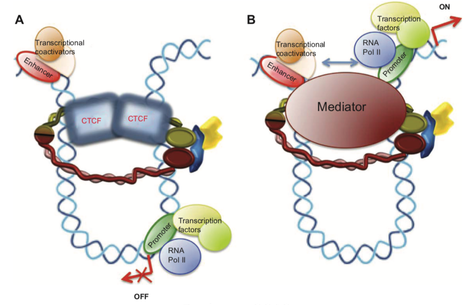
INTRODUCTION AND BACKGROUND



Horsfield et al., 2012

The cohesin complex is formed by a multi-subunit core and their associated regulatory proteins. Genetic variants within components of the cohesin complex (*NIPBL*, *SMC1A*, *SMC3*, *RAD21*, *HDAC8*) are believed to be responsible for Cornelia de Lange Syndrome (CdLS), a rare multiple malformation syndrome affecting almost any organ, in particular central nervous system (CNS) and causing severe developmental delay. The cohesin complex has a canonical role in cell division and a non-canonical role in gene expression regulation. "Cohesinopathies" seem to be caused by dysregulation of specific pathways arising from mutations in cohesin components, and canonical WNT pathway appears to be the most relevant for a proper neurodevelopment.

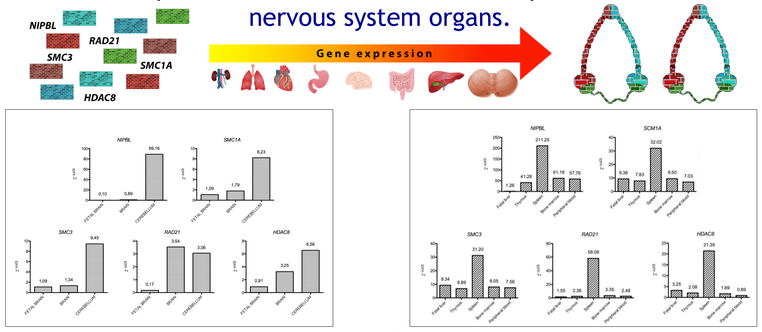
Patients affected by Cornelia de Lange Syndrome are characterized by slow growth before and after birth leading to short stature; intellectual disability and abnormalities of bones in the arms, hands, and fingers. CdLS patients also have distinctive facial features: synophrys, long eyelashes, low-set ears, small and widely spaced teeth, and a small and upturned nose.



Barbero, 2013

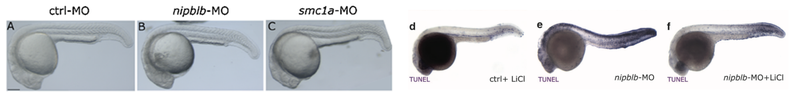
COHESIN EXPRESSION

Using both quantitative and qualitative methods in mammalian fetal and adult tissues, we have previously shown that cohesin genes are ubiquitously and differentially expressed. In particular, abundant expression was observed in hematopoietic and central nervous system organs.



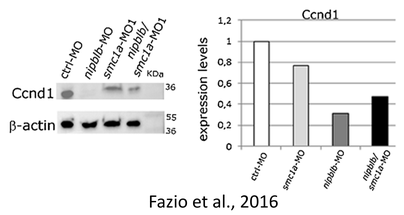
Bettini et al., 2018

ZEBRAFISH



Fazio et al., 2016 — Pistocchi et al., 2013

Zebrafish morphants for *nipblb* and *smc1a* display phenotype dysmorphism and increased cell death, especially in the CNS. Both these features have been restored activating WNT pathway treating fish with 0,3 M dose of lithium chloride (LiCl).



Fazio et al., 2016

CyclinD1 (*ccnd1*) was downregulated in both morphants. This could explain cell death since its importance during cell cycle, and our data further confirm the relevance of WNT pathway in this process.

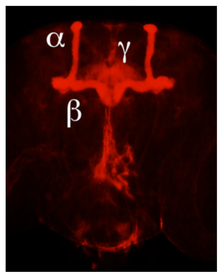
DROSOPHILA

	Common food	Food added with LiCl
LiCl 50mM ✓		
LiCl 100mM ✓		
LiCl 200mM ✓		
LiCl 500mM ✗		
yw ♂	0,7440 mg	*not enough flies*
yw ♀	1,2360 mg	1,2222 mg
nipped-B ♂	0,7141 mg	*not enough flies*
nipped-B ♀	1,1780 mg	1,1571 mg

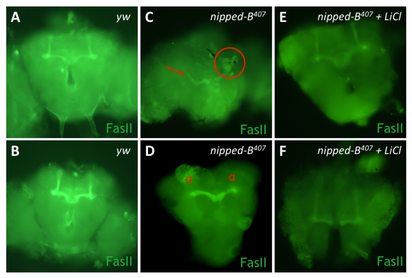
nipped-B in *D. melanogaster* is the ortholog of the human *NIPBL* gene. nipped-B interacts with mau2 to load the cohesin ring complex onto chromosomes. nipped-B and cohesin participate in transcriptional regulation and DNA repair.

We are testing the mutated loss-of-function allele ***nipped-B^{407}***. *nipped-B^{407}* mutants are known to possess fewer cells with a smaller size in the adult stage, therefore these mutants' weight is lighter (Wu et al., 2015). Our data showed a non significant reduction in mutants weight hence this assay is not suitable for drug testing.

nipped-B^{407} mutants display mushroom bodies (MB) malformations, a structure involved in olfactory learning and memory. Upon treating adult flies with LiCl, we observe a rescued morphology in the offspring's MB. 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.



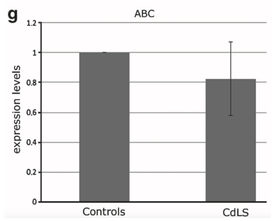
Wu et al., 2015



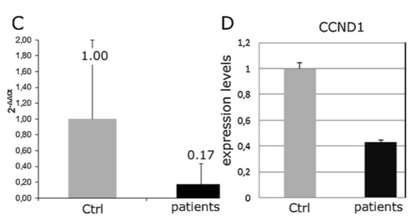
Unpublished data

PATIENTS' FIBROBLASTS

Biopsies from mutated patients in *NIPBL* and *SMC1A* were used in these studies. Skin fragment were cultured in RPMI medium supplemented with 20% fetal bovine serum after being collected and shredded in sterile conditions. These cells display downregulation of active β-catenin (ABC) and CyclinD1 (CCND1). This confirms canonical WNT pathway impairment in a CdLS model.

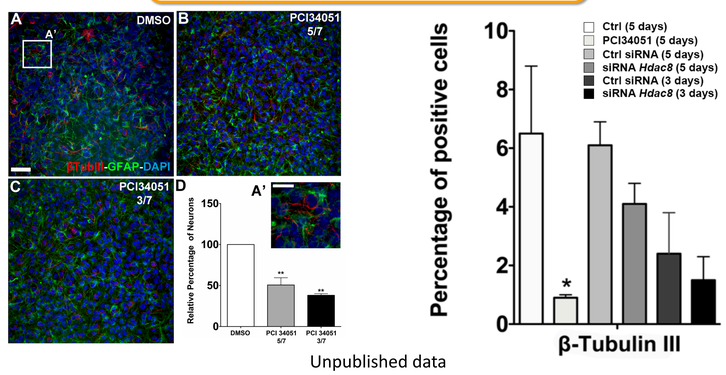


Pistocchi et al., 2013



Fazio et al., 2016

MURINE NSCs



Unpublished data

NSCs treated with PCI34051 (chemical compound that specifically inhibits HDAC8) or injected with siRNA against *hdac8* show reduced NSCs proliferation rate and differentiating capabilities.

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There is no conflict of interest to be disclosed

This work has been supported by Fondazione Cariplo, grant 2015-0783 to Valentina Massa and PhD program in Molecular and Translational Medicine - University of Milan scholarship to Paolo Grazioli