LHX2 loss of function causes neurodevelopmental deficits in humans and flies

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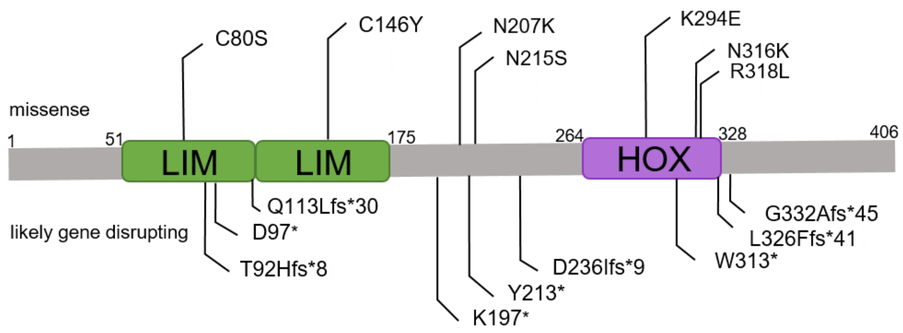
Summary

Neural development is a highly complex process with many different genes involved in activation and repression of transcriptional pathways. An essential player in this process is the LIM homeobox 2 gene (*LHX2*), coding for a well conserved transcription factor and sharing similar functions across species.
Through exome sequencing and international collaboration, we identified 16 individuals with truncating or missense variants in *LHX2* and intellectual disability of variable degrees. All but one of the variants occurred *de novo*. Additionally to the intellectual disability, the affected individuals presented with microcephaly and behavioural anomalies including sleeping difficulties.
While a loss-of-function mechanism is likely for the truncating variants, we wanted to further investigate the functional consequences of the missense variants. Using immunofluorescence, we found nucleolar accumulation for two mutants localized to the HOX domain, which mediates DNA binding. Furthermore, using co-immunoprecipitation analysis, an impaired interaction with LDB1 was observed for one LHX2 mutant located in the LIM domain, which mediates protein-protein interaction. These results suggest a loss-of-function mechanism for the missense variants as well.
To model the impact of LHX2 loss on neuronal function and behaviour, we investigated effects of pan-neuronal knockdown of *LHX2* orthologue *apterous* in *Drosophila melanogaster.* We found impaired basic locomotor ability in the climbing assay as well as reduced daily activity and prolonged sleep cycles in the activity assay. These observations underline the importance of *apterous* in nervous system development and function and thus reinforce a role of the human orthologue LHX2 in neurodevelopmental disorders.

From variant to clinic

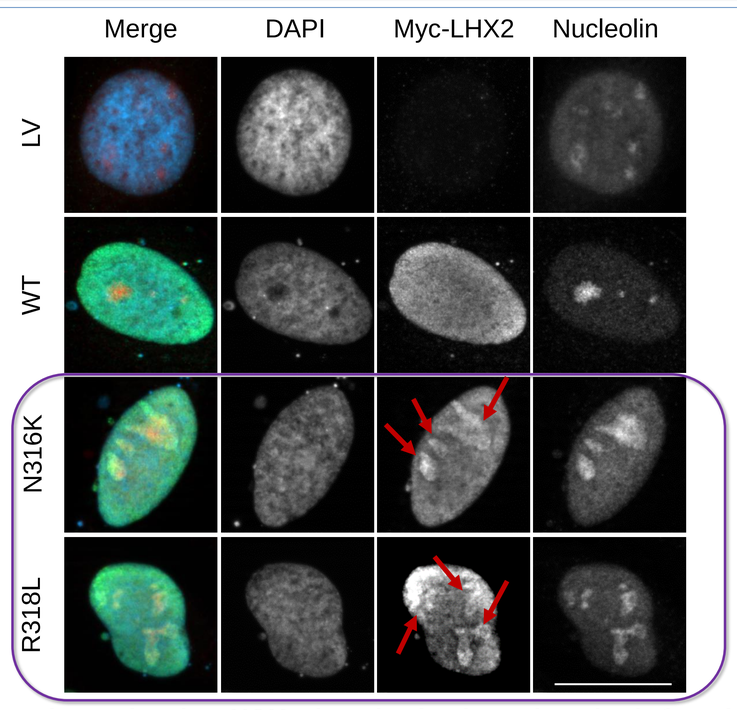


All variants are either likely pathogenic or pathogenic according to ACMG criteria.
Missense variants are on top, likely gene-disrupting variants in the bottom row. Affected individuals show variable degrees of intellectual disability, behavioral anomalies, sleeping problems and microcephaly. We found no genotype-phenotype correlation between the likely gene disrupting and missense variants.

Summary of phenotypic data

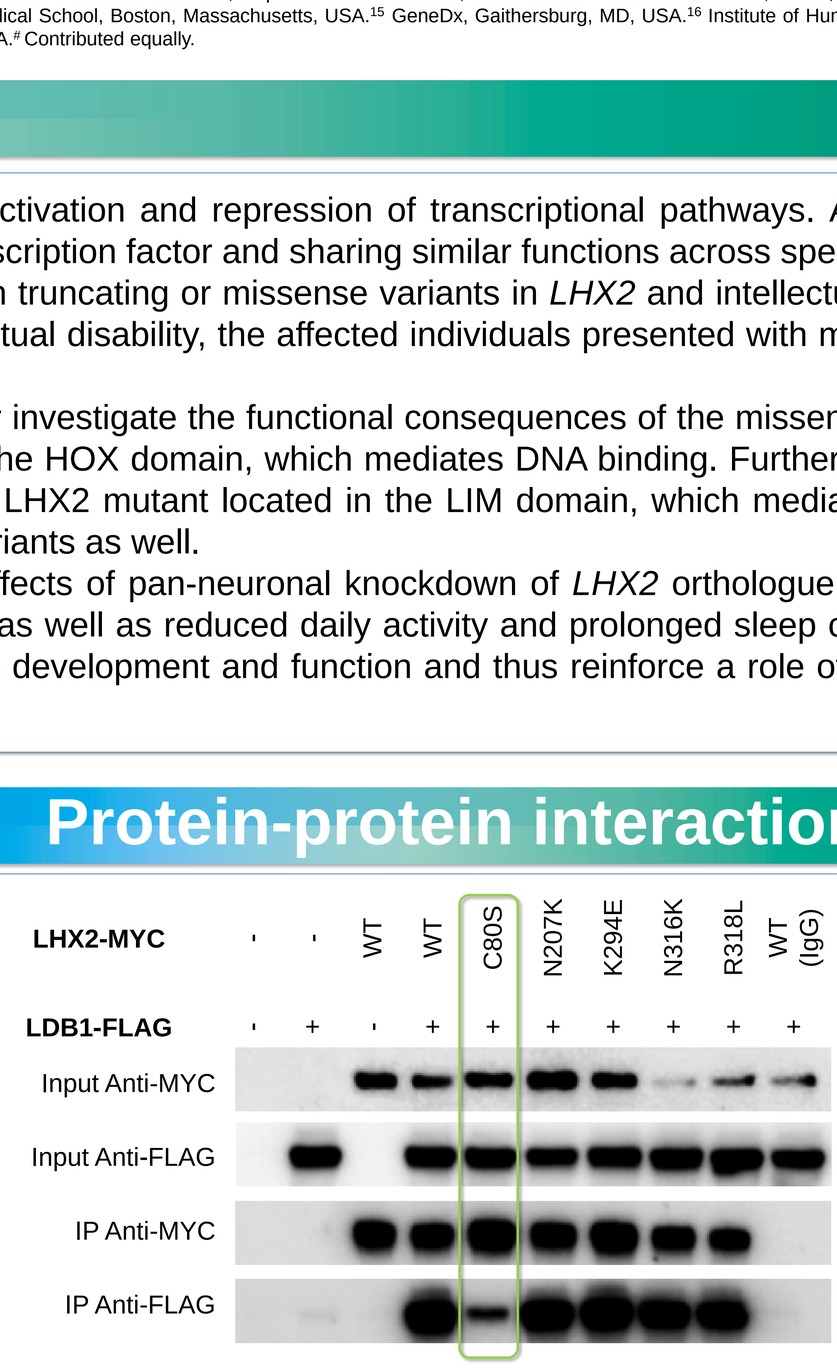
Variant Type	Likely gene disrupting	Missense	All
n	*n*=9	*n*=7	*n*=16
ID/DD	6/6	5/5	11/11
Seizures/abnormal EEG	1/3	1/4	2/7
Hypotonia	2/4	1/3	3/7
Facial dysmorphism	4/5	5/5	9/10
Microcephaly	4/6	2/4	6/10
Behavioral anomalies (incl. ASD)	4/6	6/6	10/12
Speech impairment	3/3	4/5	7/8
Feeding difficulties	2/4	1/1	3/5
Sleeping difficulties	2/3	3/6	5/9
Vision impairment	5/8	3/4	8/12
MRI anomalies	3/4	3/4	6/8

Intracellular localization



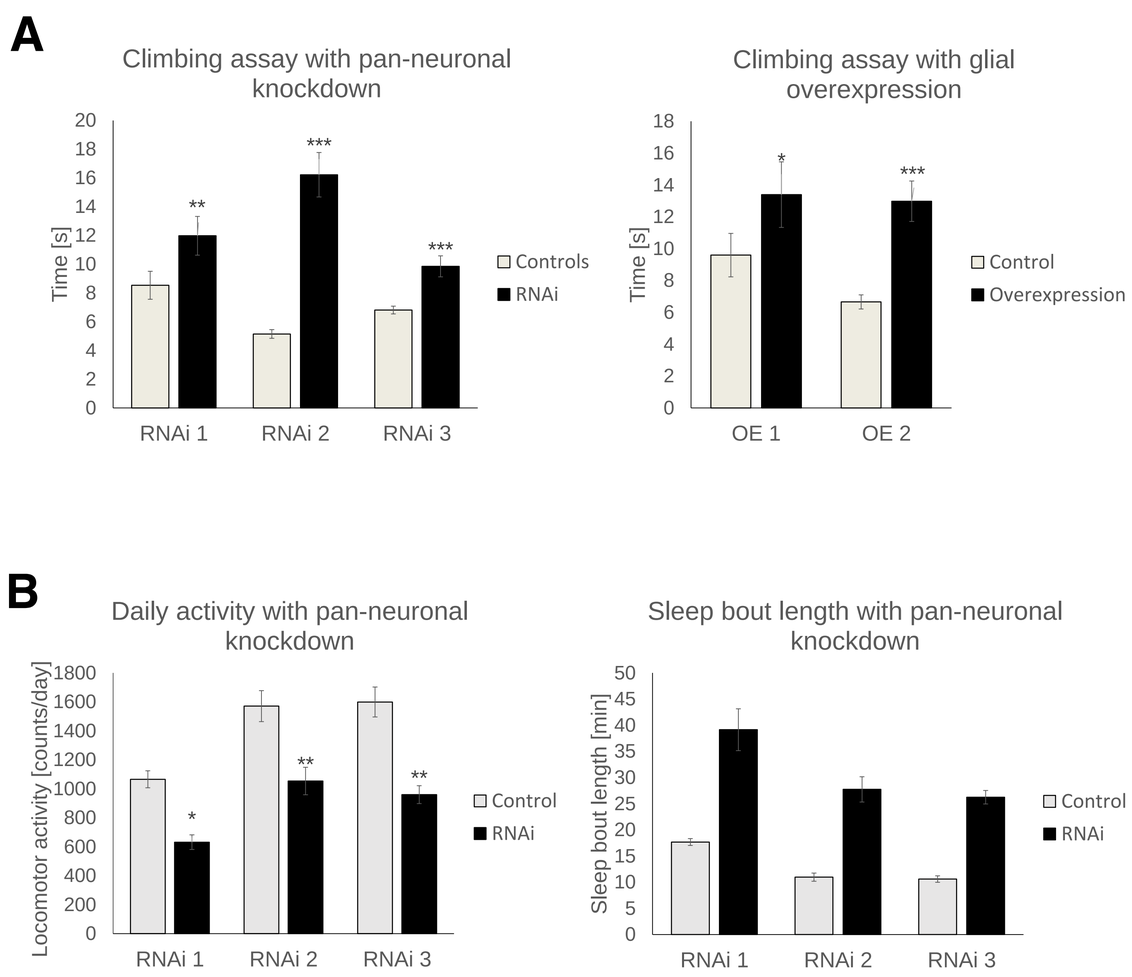
Two missense variants in the HOX domain lead to impaired function via nucleolar accumulation of LHX2. Immunofluorescence of HeLa cells transiently transfected with Myc-tagged wildtype or mutant LHX2 and co-stained with Nucleoin. Mutant protein p.(Asn316) and p.(Arg318Leu) formed aggregates in the nucleolous (red arrows), inhibiting the ability of LHX2 to bind DNA and act as a transcription factor.

Protein-protein interaction



A missense variant in the protein binding LIM domain impairs protein-protein interaction. With co-immunoprecipitation of Myc-tagged wildtype or mutant LHX2 and Flag-tagged LDB1 or their respective negative controls, we saw reduced co-precipitation of Flag-tagged LDB1 with LHX2 carrying the variant p.(Cys80Ser) in the LIM domain. This implicates the inability of the mutant LHX2 to form protein complexes with LDB1 necessary for transcriptional regulation in various stages of neural development.

Behavioral impacts in *Drosophila*



Dosage variation of LHX2 orthologue apterous leads to impaired behavioral patterns in Drosphila melanogaster. We found that pan-neuronal knockdown (RNAi 1, 2, and 3) or glial overexpression (OE1 and OE2) of *apterous* show a significantly impaired basic locomotor behavior in the climbing assay (A). Additionally, we could demonstrate that a knockdown of *apterous* lead to decreased spontaneous movement in the activity assay. Furthermore, the flies with *apterous* knockdown exhibited an increased sleep bout length (B). These results underline the importance of LHX2 in neural development and show how this well conserved gene shares similar functions among different species.

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