Trisomic dosage imbalance exhibits tissue, temporal, and sex specific non-linear genetic expression in a Down syndrome mouse model

Randall J. Roper¹, Laura E. Hawley¹, and Charles R. Goodlett² Departments of Biology¹ and Psychology² Indiana University-Purdue University Indianapolis Contact email: rjroper@iupui.edu

<image>

Background

- Trisomy 21 causes Down syndrome (DS) in humans
- Trisomic gene dosage imbalance causes DS phenotypes
- Three copy as compared to two copy genes expected to be upregulated 1.5 fold in every cell (see right)
- Dyrk1a = dosage sensitive gene important in cognitive and skeletal DS phenotypes
- Reducing *Dyrk1a* to normal copy number at conception in otherwise trisomic mice normalizes some DS cognitive and skeletal phenotypes
- Reduction of DYRK1A activity is a therapeutic goal to correct DS phenotypes

Motivation and Hypothesis

 While trying to normalize DYRK1A protein expression in DS mouse models, we found that DYRK1A expression was not upregulated 1.5-fold as expected at ~2 months in Ts65Dn mice (see right)

^{1.5}

■ Eu+Water (n=9) ■ Eu+EGCG (n=9) ■ Ts+Water (n=5) ■ Ts+EGCG (n=7)

- We wanted to normalize DYRK1A expression only when it was upregulated
- Expression levels of trisomic *Dyrk1a* not well known, especially during development
- We hypothesized that DYRK1A expression is temporally and spatially regulated in DS mouse models

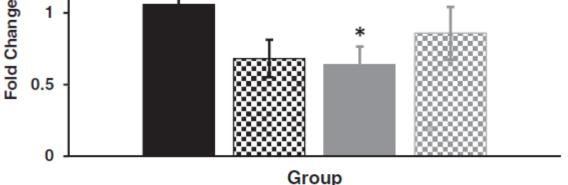
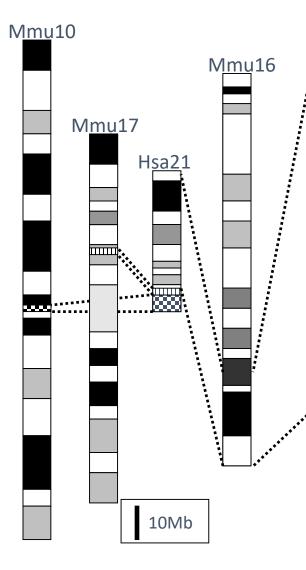


Fig. 5. Dyrk1a Protein Levels-Cerebellum. Dyrk1a protein levels in the cerebellum of \sim 9-week old mice given 6 weeks of treatment with water or EGCG (mean ± SEM). Ts65Dn mice receiving water had significantly less Dyrk1a protein that euploid-water controls (as indicated by the *, genotype × treatment interaction, p = 0.043).

Stringer et. al. *Physiol Behav*. 2017;177:230-241. doi: 10.1016/j.physbeh.2017.05.003. PMID: 28478033

Ts65Dn DS mouse model

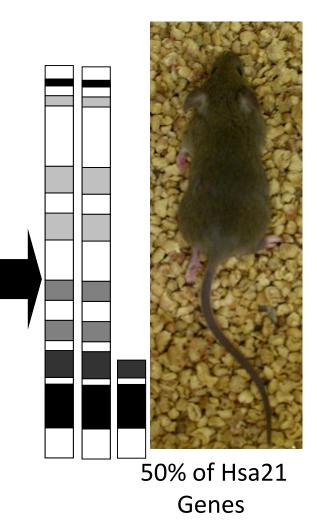


NE355P EGE7L C21orf110 GTE2IP2 ORLE ZNF299P EEF1A1P TUBAP RPL13AP C21orf42 C21orf7 JAM2 FDXP2 ATP5A GABPA APP C21orf118 C21orf79 MACSL CYYR1 ADAMTS1 ADAMTS5 GPXP2 EIF4A1P RPL10F C21orf23 C21orf94 C21orf100 DNMTA1 HSPDP7 ZNF294 C21orf98 RPL23P2 C21orf6 USP16 CCT8 C21orf109 GAPDP14 BACH1 C21orf12 C2 C21orf9 CLDN17 CLDN8 RPL8P2 KRTAP23-1 C21orf41 KRTAP13-2 KRTAP13-1 KRTAP13-3 KRTAP13-KRTAP15-1 KRTAP19-1 KRTAP19-2 KRTAP13A KRTAP19-3 KRTAP19-4 KRTAP19-5 KRTAP19A KRTAP19B KRTAP190 KRTAP19-7 KRTAP6-3 KRTAP6-2 KRTAP6-1 KRTAP20-1 KRTAP20-2 KRTAP19D KRTAP21A KRTAP21-1 KRTAP8A KRTAP8B KRTAP8 -1 KRTAP7 1 KRTAP11-1 UBE3AP2 TIAM1 D21S2086 FBXW1BP BTRC2F SOD1 CTDBP HMG14P TPT1P HUNK C21orf44 C21orf45 C21orf6 C21orf119 C21orf63 C21orf47 C21orf77 C21orf59 OR7E92P SYNJ1 C21orf66 C21orf49 C21orf120 RED36 OLIG2 OLIG1 C21orf54 IFNAR2 IL10RB IFNAR IFNGR2 C21orf4 RPS5L C21orf55 GART SON DONSON CRYZL ITSN1 ATP50 SLC5A3 MRPS6 RPS5P2 PRED37 C21orf82 KCNE2 PRED38 KCNE1 DSCR1 PRED39 CLIC6 C21orf52 RUNX1 C21orf96 RPL34P3 RPS20P PPP1R2P2 RPL23AP3 C21off18 RIMKLP C21off27 CBR1 C21off19 RPS9P CBR3 C21off5 RPL3P RPS26P SFRS9P1 KIAA0136 CHAF1B ATP5J2LP CLDN14 PSMD4 SIM2 HLCS DSCR6 DSCR5 TTC3 DSCR9 DSCR3 DYRK1A CNJ6 DSCR4 DSCR8 DSCR10 KCNJ15 ERG C21orf24 TS2 CZ1orf104 RPL23AP5 PCBP2P1 DSCR2 WDR9 C21orf107 C21orf87 HMGN1 WRB C21orf13 SH3BGR C21orf88 B3GALT5 IGSF5 PCP4 DSCAM FLJ37173 BACE2 C21orf75 C21orf15 FAM3B MX2 MX1 TMPRSS2 C21orf20 C21orf21 FLJ32835 21orf22 ANKRD3 PRDM15 C21orf25 ZNF295

COL18A1 C21orf123 SLC19A

SS MCM3APAS MCM3AP C21orf57 C21orf58 PCNT

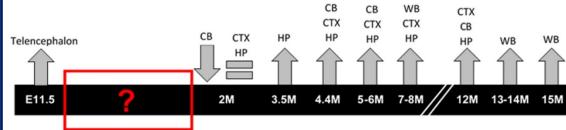
IAA0184 S100B HRMT1L1 RPL23AP4



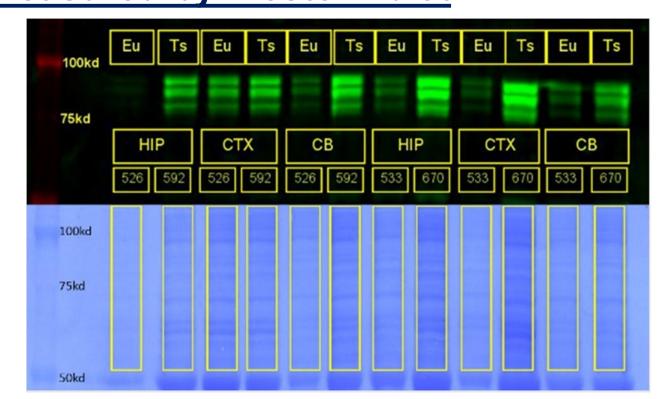
- Most common used DS mouse model
- Three copies of 104 genes that are found on Mmu16 and homologous to Hsa21
- Extra genes on a freely segregating chromosome
- Display cognitive, behavioral, and skeletal, phenotypes similar to humans with DS

• Has three copies of *Dyrk1a*

Unknown period of DYRK1A expression measured by Western blot



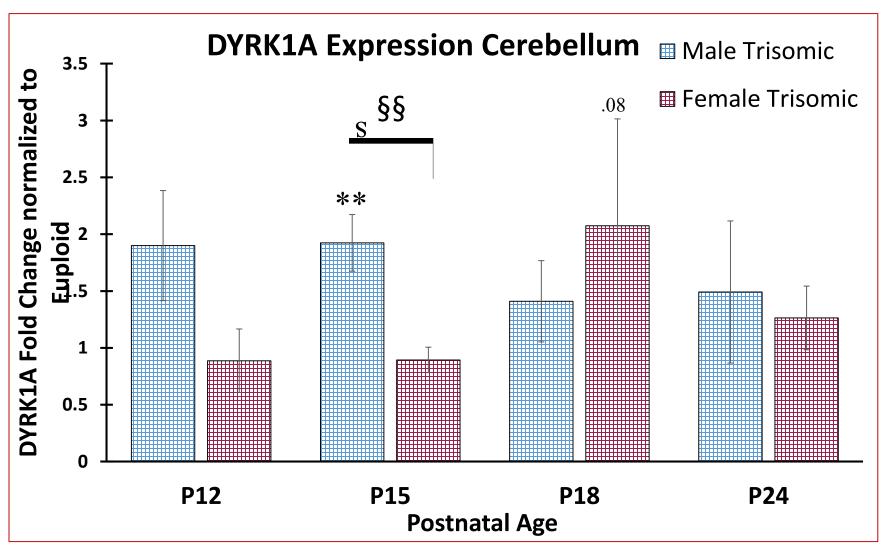
Others have measured DYRK1A expression in mouse models during different developmental times in different brain regions.
We measured DYRK1A protein expression of in 3-6 day windows during development in cerebellum (CB), hippocampus (HIP) and cerebral cortex (CTX) of Ts65Dn and control littermate mice



- Top: DYRK1A antibody (M01, clone 7D10, Abnova)
- Bottom: Coomassie-stained membrane (same membrane as above)
- Protein from trisomic and euploid littermates for each brain region loaded in adjacent lanes.

Temporally, spatially and sex specific DYRK1A expression

- DYRK1A protein expression in Ts65Dn as compared to normal brains varies during development from 1- to 5fold, showing dosage level compensation, expression, and amplification of DYRK1A protein varies according to tissue (only cerebellum shown)
- DYRK1A expression varies according to sex—notice the difference in expression at P15 between males and females



P12=Postnatal Day 12, etc.

** = P < .01 relative to euploid control

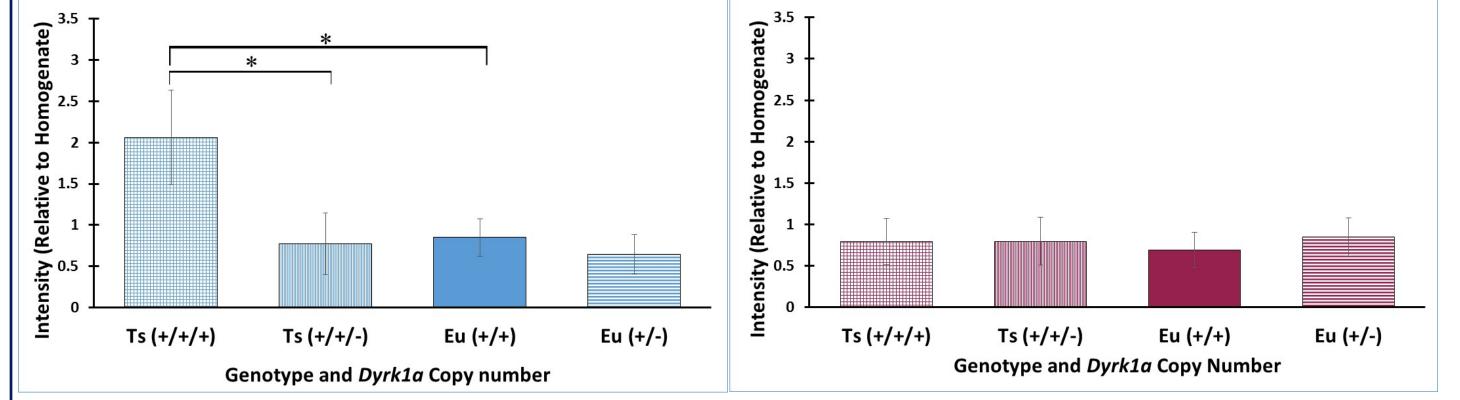
§§ = P < .01 difference between sexes

DYRK1A expression in genetically modified mice

DYRK1A expression in P15 Ts65Dn (3 copies *Dyrk1a*), Ts65Dn +/+/- (2 copies of *Dyrk1a*), Euploid (2 copies of *Dyrk1a*), and Euploid +/- (1 copy of *Dyrk1a*) mice

Variable Dyrk1a Dosage Male Cerebellum

Variable Dyrk1a Dosage Female Cerebellum



- In P15 males where DYRK1A was overexpressed, expression is downregulated with one fewer copy
- In P15 females where DYRK1A was not overexpressed, expression is the same in Ts65Dn, Ts65Dn +/+/-, and Euploid mice

Conclusions

- Trisomic DYRK1A expression varies during development from the expected 1.5fold level of expression, some regions have high overexpression, other regions have no overexpression or dosage compensation
- DYRK1A trisomic expression in brain varies according to age, sex, and tissue type
- DYRK1A temporal and tissue specific expression may or may not correlate with gene dosage of *Dyrk1a* found in the tissue
- These data suggest that DYRK1A expression is controlled at the transcriptional, translational, or post-translational level, perhaps by other trisomic genes
- To optimize the effectiveness of potential therapies to reduce activity of DYRK1A, treatments should target the tissue-specific developmental periods of overexpression.

This project is supported by the Indiana Clinical and Translational Sciences Institute and funded in part by Grant Number UL1TR001108 from the National Institutes of Health, National Center for Advancing Translational Sciences, Clinical and Translational Sciences Award. Additional funding was provided by a Research Support Funds Grant and the Department of Psychology at IUPUI.