Modelling IRF2BPL-related Pediatric Neuroregression in Mice

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INTRODUCTION

De novo truncating variants in the gene *IRF2BPL* cause severe childhood-onset ataxia termed NEDAMSS (Neurodevelopmental disorder with abnormal movements, loss of speech and seizures). Since 2018, over thirty cases have been published. Additionally, *IRF2BPL* missense variants are associated with autism spectrum disorder. Little is known about IRF2BPL function, but it is important in nervous system development and maintenance. Using *Drosophila* models, the fly ortholog plays an important role in neuronal maintenance. It has been recently shown that loss or gain of IRF2BPL leads to excess or decreased Wnt transcription, respectively. In addition, loss of IRF2BPL leads to neurodegenerative phenotypes in flies and zebrafish. In order to further understand the protein, we developed a model for preclinical testing by generating the first *Irf2bpl* knock-out mice.

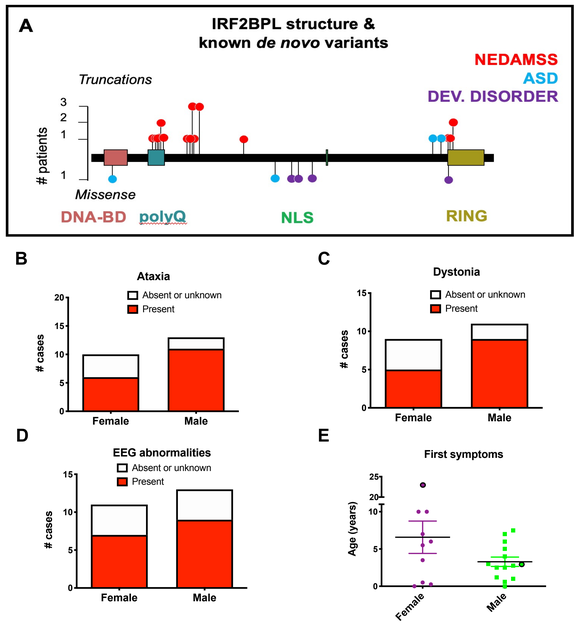

Figure 1: IRF2BPL-associated disease.
(A) IRF2BPL protein structure with *de novo* nonsense (top) and missense (bottom) variants associated with disease. Gene constraint data from ExAC control database indicates IRF2BPL is highly constrained. (B-E) Presence of ataxia, dystonia, EEG abnormalities and age at first symptoms in NEDAMSS.

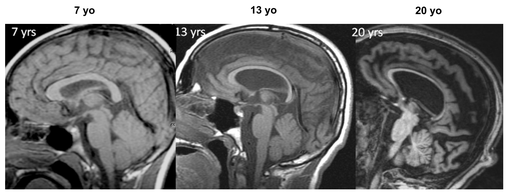

Figure 2: NEDAMSS patient exhibit progressive CNS atrophy.
Progressive cerebral and cerebellar atrophy in individuals with IRF2BPL nonsense truncations. Brain MRI for left subject (axial FLAIR) at years indicated.

AIM

We generated the first *Irf2bpl* knockout mice and hypothesize that *Irf2bpl* heterozygous mice show deficits in motor function, mimicking the putative haploinsufficiency observed in patients with NEDAMSS.

METHOD

We generated an *Irf2bpl* null allele by removal of the majority of the coding region of the intronless, *Irf2bpl* gene (Δ17-651). We performed heterozygous crosses to assess viability, mass, and motor function by vertical pole test and inverted grid test in wild-type (WT), heterozygous (HET), and homozygous (KO) littermates.

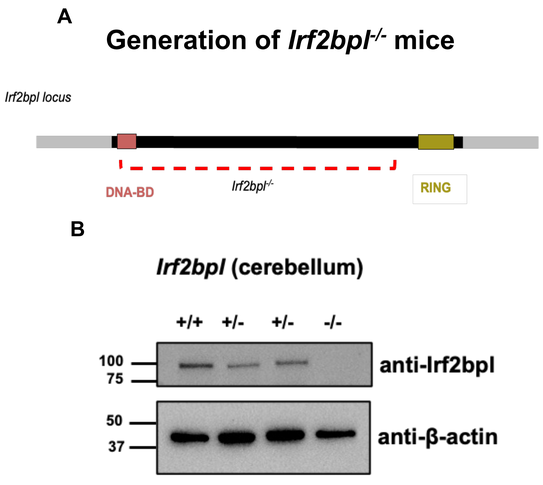

Figure 3: Generation of Irf2bpl knockout mice.
(A) Schematic of *Irf2bpl* locus in mice and *Irf2bpl* KO allele. (B) Absence of Irf2bpl protein in *Irf2bpl* KO mouse cerebellum by western blot.

RESULTS

We observed that *Irf2bpl* KO mice are born at lower Mendelian ratios, close to 33% of expected animals survive until weaning. Although WT and HET mice did not have a significant difference in mass, KO mice were 68% +/- 4.2% (standard error of the mean) for males and 76% +/- 5.6% (SEM) for females at three months of age. Three-month-old *Irf2bpl* KO mice display motor defects on the vertical pole test and inverted grid test. Specifically, *Irf2bpl* KO mice took about 1.7X longer than WT or HET mice to descend a vertical pole and half of the KO mice failed to hang onto the inverted grid where nearly all WT and HET mice could successfully hang onto the grid.

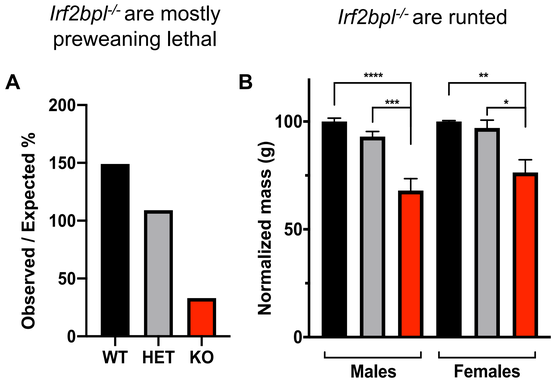

Figure *4: Irf2bpl*$^{-/-}$ mice are sub-viable and runted
(A) *Irf2bpl* KO mice had lower Mendelian ratios due to preweaning lethality. (B) *Irf2bpl* KO mice had decreased body mass compared to littermates of the same sex. ANOVA followed by Tukeys. Results are means ± SEM (*P < 0.05, **P < 0.01, ***P < 0.001, ****P < 0.0001).

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Irf2bpl KO mice display motor phenotypes at 3 months

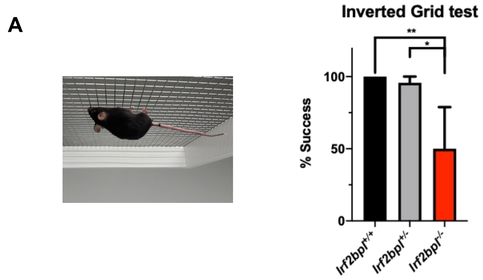

Figure 4: KO mice display phenotypes
Irf2bpl KO display increased failures, falling off the inverted grid (A) and increased time descending the vertical pole test (B). ANOVA followed by Tukeys. Results are means ± SEM (*P < 0.05, **P < 0.01).

CONCLUSION

We hypothesized *Irf2bpl* HET mice would display motor phenotypes, but we only observed motor deficits in KO mice. The *Irf2bpl* KO mice display motor defects reminiscent of NEDAMSS and may serve as an informative model.

FUTURE DIRECTIONS

Aging studies should be performed to determine if *Irf2bpl* HET mice display progressive behavioural phenotypes at later stages. More in depth behavioral studies should be employed as well as characterizing brain pathology in young and aged mice.

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