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THE GENETIC UNDERPINNINGS OF GORDON HOLMES SYNDROME

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INTRODUCTION

Gordon Holmes Syndrome (GDHS) is a rare, but fatal autosomal recessive neurodegenerative disorder. It causes symptoms of cerebellar ataxia, neuroendocrine failure, and commonly dementia. Following a normal childhood development, symptoms appear during the teenage years and continue to develop in a progressive nature. Typically, patients find themselves bed-ridden by their twenties and most die by their mid-forties. Recently a study used whole exome sequencing that linked mutations in the E3 ligase RNF216 to the pathogenesis of the disease [1]. However, despite identifying this molecular basis, the biological mechanism remains elusive. Thus, our objective was to develop a zebrafish model of GDHS and focus on the cerebellum and hypothalamus to determine mechanisms that lead to ataxia and neuroendocrine failure, respectively.

METHODS

Morpholino (MO) microinjections were used to knockdown the *rnf216* gene in zebrafish embryos. Embryos were separated into three treatment groups: uninjected controls, vehicle controls, and MO treated (injected with concentrations of 1.6 or 5.0ηg). Upon sacrifice at 48hpf, 3dpf, 5dpf, and 7dpf, immunohistochemistry was used to look at the molecular markers SV2, which marks synaptic vesicles containing neurotransmitters, zebrinII, which marks purkinje cells responsible for the regionalization of the cerebellum, the apoptotic marker caspase3, and sox2 for progenitor cells. In situ hybridizations on 3dpf zebrafish were also performed using hypothalamic riboprobes *nkx2.1a*, *hypocretin*, and *vsnp*.

RESULTS

Our data show increased SV2+ neurons in the cerebellum of 1.6ng MO1-*rnf216* injected zebrafish at 3dpf (Fig. 1). These morphants also show a decreased neuronal population in the pan-hypothalamus, indicating developmental dysregulation. These results imply that defects occur in a time specific manner in our zebrafish model of Gordon Holmes Syndrome, which is also morpholino concentration dependent. As well, consistent with an increased cell population (SV2+ neurons), cell death was not present at this timepoint and no differential expression was observed for the apoptotic marker anti-caspase3. Preliminary data also indicate morphants have decreased anti-zebrinII, anti-sox2 and differential *hypocretin* and *vsnp* expression.

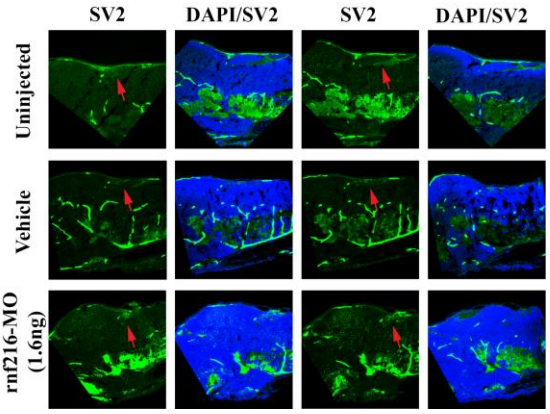

Figure 1. Serial sagittal sections of the cerebellum (7μm thickness) show SV2+ neuronal elements (green) and the outline of the tissue was obtained using the nuclear stain, DAPI (blue). SV2+ neurons are increased in the cerebellum of rnf216-MO injected zebrafish (1.6ηg) when compared to vehicle and uninjected controls (highlighted with red arrowheads).

DISCUSSION AND CONCLUSIONS

Increased SV2+ neurons in the cerebellum of 1.g *rnf216* MO-injected zebrafish at 3dpf suggests a dysregulation of synaptic pruning early in development of these mutants. Synaptic pruning is an essential process that refines neuronal circuitry by removing unnecessary and unwanted connections in order to strengthen more productive ones. Though cellular and molecular mechanisms are not very well understood, studies have indicated the role of E3 ubiquitin ligases, such as RNF216, in regulating the process of synaptic pruning [2]. Hence a mutated RNF216 may directly be linked to the increased synapses present, as it may not properly function to remove the unwanted connections. Combined these findings start to provide insight into the etiology behind this disease and to further understand the role of RNF216 in implicating cerebellar ataxia and neuroendocrine dysregulation.

REFERENCES

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2. Chia PH, et al. *J Cell Biol*. **203**(1): 11-22, 2013.