

# Investigating the efficacy of combined ketogenic diet and anti-inflammatory therapy in a mouse model of early-onset Tay-Sachs disease

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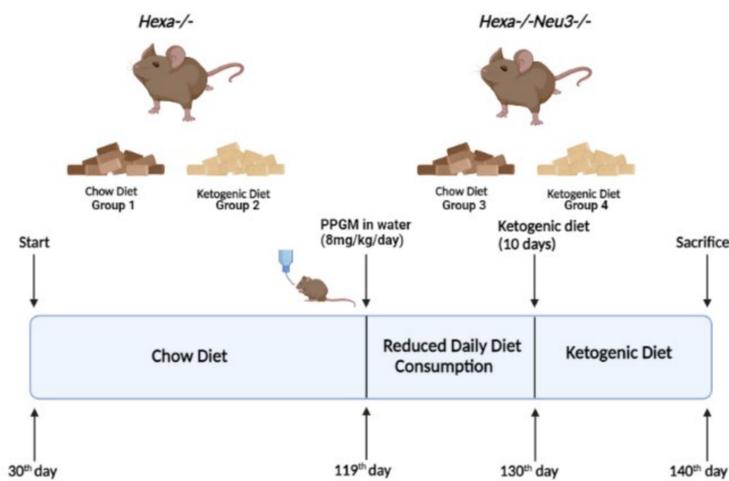
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## INTRODUCTION

Tay-Sachs disease is a rare genetic disorder caused by abnormal GM2 ganglioside accumulation predominantly in the central nervous system (CNS) due to  $\beta$ -hexosaminidase A (HexA) enzyme deficiency. HexA enzyme mediates GM2 ganglioside to GM3 ganglioside conversion in the cell. In human, infants are healthy at birth but, progressive accumulation of GM2 ganglioside causes disruption of motor functions, inducing neuronal cell death. Unlike infants, *Hexa*<sup>-/-</sup> mouse model did not show severe pathology due to metabolic bypass mechanism for GM2 ganglioside degradation. Recently, our research group generated *Hexa*<sup>-/-</sup>*Neu3*<sup>-/-</sup> mouse model that mimics neuropathology of early-onset Tay-Sachs disease (1). Novel role of Neu3 sialidase in the ganglioside degradation was discovered by this way. We showed that undegraded GM2 accumulation resulted in neuronal cell death and activated neuroinflammation inducing astrogliosis and microgliosis-based pro-inflammatory cytokines and chemokines secretion such as Ccl2, Ccl3, Cxcl10 (2). Ccl2 cytokine is a type of neuroinflammation mediators in the neuroinflammatory disorders. Astroglial and microglial expression of Ccl2 cytokine elevates in the site of neuronal injury (3).

The high fat, low-carbohydrate ketogenic diet (KD) has broad potential usage in the treatments of neurological disorders and there is growing evidence that KD is also anti-inflammatory (4). In addition to ketogenic diet, propagermanium (Ccl2/Ccr2 axis inhibitor) is used to block Ccl2/Ccr2 axis to reduce inflammatory responses from microglia and astrocyte (5). In this study, we aim to show KD and anti-inflammatory drug therapy in the treatment of neuroinflammation in *Hexa*<sup>-/-</sup>*Neu3*<sup>-/-</sup>.

## METHODS



**Figure 1:** Illustration of the time schedule of combined ketogenic diet and propagermanium therapy for *Hexa*<sup>-/-</sup> and *Hexa*<sup>-/-</sup>*Neu3*<sup>-/-</sup> mouse model.

### Real-Time PCR:

RNA was isolated from cortex and cerebellum of *Hexa*<sup>-/-</sup> and *Hexa*<sup>-/-</sup>*Neu3*<sup>-/-</sup> mice for analysis of neuroinflammation markers. Expression levels of neuroinflammation markers (Ccl2, Ccl3, Ccl5, Cxcl10, GFAP) were analyzed by using SYBR Green I Master Mix. GAPDH gene expression was used as control.

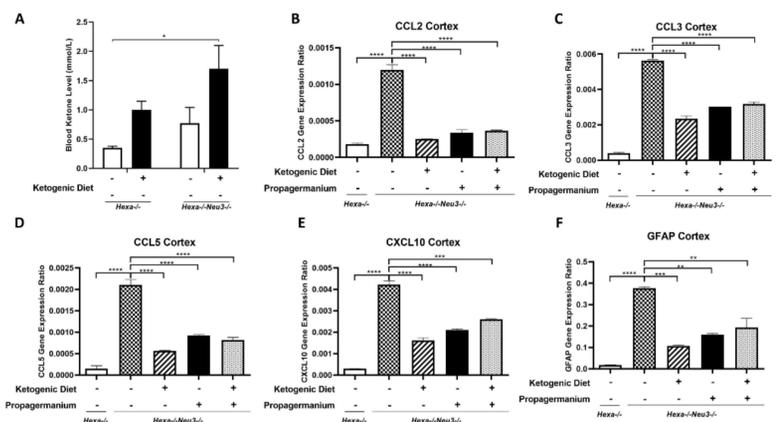
### Immunohistochemistry:

Mice were anesthetized and perfused with 4% paraformaldehyde (PFA) in phosphate buffer saline (PBS). The 10  $\mu$ m brain sections from the mice at the indicated ages were stained with anti-GFAP (glial fibrillary acidic protein; Sigma, Germany) was diluted to 1:200 in blocking buffer and applied overnight at 4 °C. The binding of GFAP was visualized using goat antimouse Alexa Fluor 488 antibody. The slides were mounted with Fluoroshield mounting medium with DAPI (Abcam, USA) and images were obtained using fluorescence microscopy (Olympus, Germany).

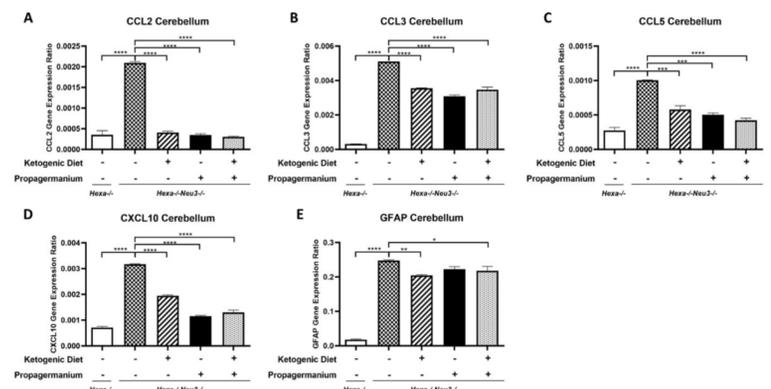
## ACKNOWLEDGEMENT

The research was supported by TUBITAK grant no:218S824 and COST Action-CA17103.

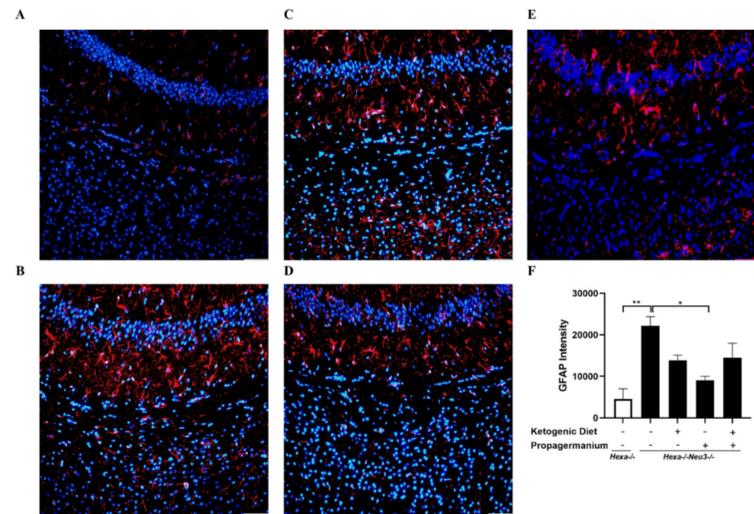
## RESULTS



**Figure 2:** Measurements of blood ketone levels (mmol/L) for *Hexa*<sup>-/-</sup> and *Hexa*<sup>-/-</sup>*Neu3*<sup>-/-</sup> mice (A) (n=3). The expression levels of neuroinflammation markers named as Ccl2 (B), Ccl3 (C), Ccl5 (D), Cxcl10 (E) and GFAP (F) in cortex of *Hexa*<sup>-/-</sup> and *Hexa*<sup>-/-</sup>*Neu3*<sup>-/-</sup> mice (n = 2). Statistical analysis was performed using one way ANOVA (\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.005, and \*\*\*\*p < 0.001).



**Figure 3:** The expression levels of neuroinflammation markers named as Ccl2 (A), Ccl3 (B), Ccl5 (C), Cxcl10 (D) and GFAP (E) in cerebellum of *Hexa*<sup>-/-</sup> and *Hexa*<sup>-/-</sup>*Neu3*<sup>-/-</sup> mice. (n = 2). Statistical analysis was performed using one way ANOVA (\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.005, and \*\*\*\*p < 0.001).



**Figure 4:** Immunostaining of GFAP in *Hexa*<sup>-/-</sup> (A), *Hexa*<sup>-/-</sup>*Neu3*<sup>-/-</sup> (B), Ketogenic diet-treated (C), Propagermanium-treated (D) and combined ketogenic diet and propagermanium-treated (E) *Hexa*<sup>-/-</sup>*Neu3*<sup>-/-</sup> mice brain cortex. The 10 $\mu$ m coronal sections were stained in red by anti-GFAP antibody (astrocyte marker). Scale bar = 50  $\mu$ m. Histogram represents astrocyte intensity in the cortex (F). The data are represented as the mean  $\pm$  SEM. One-way ANOVA was used for statistical analysis (\*p < 0.05, \*\*p < 0.01).

## CONCLUSION

In order to determine the efficacy of combined ketogenic diet and propagermanium therapy in early-onset Tay-Sachs disease mouse model, neuroinflammation-related markers were analyzed by RT-PCR and immunohistochemistry. Pro-inflammatory cytokine (Ccl2, Ccl3, Ccl5, Cxcl10) expression levels significantly decreased after combined ketogenic diet and propagermanium therapy both in cortex and cerebellum of *Hexa*<sup>-/-</sup>*Neu3*<sup>-/-</sup> compared *Hexa*<sup>-/-</sup> mouse model. In addition, astrocyte marker of GFAP expression levels significantly decreased in cortex but not efficient in cerebellum. GFAP staining displayed the similar pattern with the GFAP expression level in cortex after combined ketogenic diet and propagermanium therapy in *Hexa*<sup>-/-</sup>*Neu3*<sup>-/-</sup>.

Altogether, our results clearly suggest that combined ketogenic diet and propagermanium treatment could be potential therapeutic strategy to reduce neuroinflammation in an early-onset Tay-Sachs disease mouse model.

## REFERENCES

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