

Early Murine Embryonic Cardiac Hypertrophy Associated with Increased Ganglioside Storage

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Synopsis

In this study, MRI was utilized to evaluate the role of ganglioside, GM2, in the embryonic cardiac development in a mouse model of Sandhoff's disease.

Introduction

The enzymatic hydrolysis of the GM2 ganglioside is catalyzed by the $\alpha\beta$ -hexosaminidase (Hex) protein. There are two major isoforms of Hex: Hex A (composed of one α and one β subunit) and Hex B (composed of two β subunits). The lysosomal storage disease, Tay-Sachs disease, results from mutations of HEXA gene encoding the α subunit of Hex A. Another lysosomal storage disease, Sandhoff's disease, on the other hand, results from mutations of the HEXB gene encoding the β subunit. In both diseases, there are different levels of GM2 ganglioside accumulation related to neurodegeneration.

Most studies conducted in these lysosomal storage diseases have been focused on the central nervous system (CNS) and neurodegeneration. However, GM2 accumulation occurs in many different tissue types including cardiac muscle. Several clinical studies have shown Sandhoff's disease in humans also encompasses cardiac dysfunction. However, because GM2 accumulation is due to a genetic defect and may initiate prior to birth, it is important to trace such a cardiac phenotype change to its earliest stage with embryonic MR imaging.

Methods

Embryos (14.5-19 dpc) were collected from perfusion fixed pregnant Hexb female [4% Paraformaldehyde in phosphate-buffered saline]. After the extraction, the embryos were quickly immersed in 4% Paraformaldehyde in phosphate-buffered saline at 4°C and shook overnight. The next morning the embryos were transferred into 0.01% Azide in phosphate-buffered saline (changed every day) at 4°C and shook for one week. Each embryo was then submerged in Fomblin (Alderich, St. Louis, MO) in 5ml freestanding mailing tube (VWR, West Chester, PA) with its spine parallel with the long axis of the tube, and imaged using a Bruker Avance Biospec, 9.4 T spectrometer, 21 cm bore horizontal imaging system (Bruker Biospin, Billerica, MA) with a 35 mm volume resonator. Gradient-echo 3D sequence was applied, with TR=30.0ms, TE=3.2ms, field of view of 25.6mm*25.6mm*30.0mm, matrix size 256*256*300. 20-24 signal averages were used for each subject. Due to contrast issue, one embryo in batch 5 was imaged using RARE 2D sequence with TR=4000.0ms, TE=45.8ms, Rare Factor=8, axial and coronal cuts with slice thickness of 0.5mm, matrix size of 256*256. 4 signal averages. One axial slice was selected from each dataset which represented the largest ventricular area (usually half of the long axis). Front limbs and/or lower jaw were also used as a reference when choosing such a slice. All the rest slices in each dataset were inspected to ensure the selected slice best represent the typical septum and ventricular wall thickness.

Results:

In this study, 5 batches of embryos were imaged. Each batch came from the same litter and included at least one $-/-$. Each batch included at least one control, except for batch 4 where no wildtypes ($+/+$) were available (for this batch, one heterozygote ($+/-$) was imaged for reference). For batch 2 (17 dpc, Figure 2), the knockout ($-/-$) showed mild hypertrophy (especially on septum) compared to 3 controls (arrow). For batch 4 (14.5 dpc, Figure 4), both $-/-$ showed severe hypertrophy on septum and ventricular walls compared to the $+/-$ (arrows). A coronal view of one $-/-$ was added for a better presentation of the diminished size of the LV and RV. For batch 5 (19 dpc, Figure 5), the $-/-$ showed even more severe hypertrophy on ventricular wall and dramatically reduced ventricles (see LV and RV arrows) compared to the control. For batch 1 (17 dpc, Figure 1) and batch 3 (16.5 dpc, Figure 3), we observed no notable difference between $-/-$ and control.

Discussion: We have shown that the majority of the hexb $-/-$ embryos analyzed exhibited a cardiac deficit indicating that GM2 plays a role in embryonic cardiac development. This information is useful in that it provides information related to the mechanism of GM2 accumulation and cardiac hypertrophy. Because hypertrophy is present in the embryo, it is unlikely that the cardiac hypertrophy observed in juveniles is due to changes in blood pressure indicating the likelihood of a novel mechanism for the hypertrophic event. We are currently utilizing histology and quantitative RT-PCR to determine if the mechanism deviates from traditional cardiac hypertrophic mechanisms.

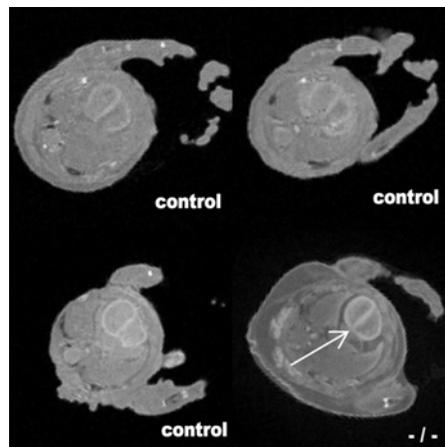


Fig.2 (17dpc)

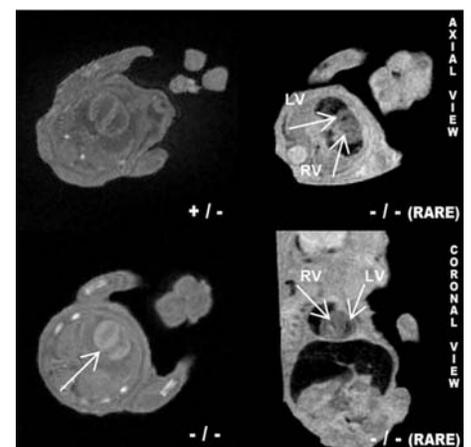


Fig.4 (14.5dpc) (contain 2 views from same $-/-$)

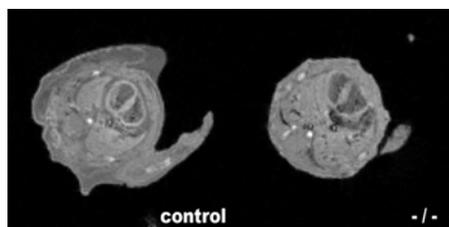


Fig.1 (17dpc)

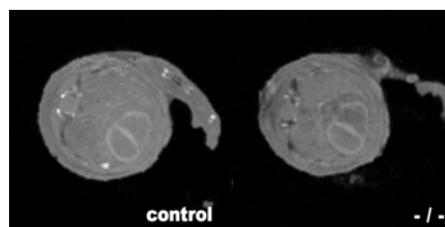


Fig.3 (16.5dpc)

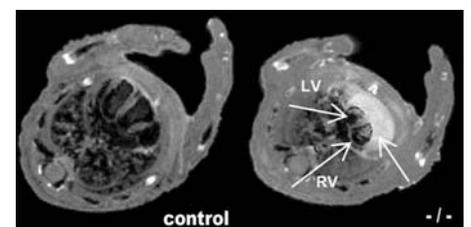


Fig.5 (19dpc)