

[712] Improved Reduction in GM2 Ganglioside Accumulation in Tay-Sachs Mice Using a New Hexosaminidase Variant

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GM2 gangliosidosis is a family of three genetic neurodegenerative disorders caused by the accumulation of GM2 gangliosides (GM2). Two of these are due to the deficiency of one of 2 similar but non-identical subunits that comprise heterodimeric β -hexosaminidase A (HexA) which hydrolyzes GM2. Mutations in the α -subunit (encoded by *HEXA*) of the enzyme HexA lead to Tay-Sachs disease (TSD), wherein mutations in the β -subunit (encoded by *HEXB*) lead to Sandhoff disease (SD). In their acute infantile forms, both rapidly progress with fatal neurological deterioration during childhood. The most significant pathological feature of TSD and SD is GM2 accumulation in neurons. Since functional HexA is a heterodimer of the α - and β -subunits, the efficacy of overexpressing only the deficient subunit in a gene therapy approach is limited by the levels of the endogenous subunit. An effective approach to treat either TSD or SD would be to express the α - and β -subunits at equimolar ratios from the same vector, which for AAV has been limited by size restrictions.

The present study used a new variant of the Hex α -subunit, containing critical sequences from the β -subunit that can form a stable homodimer (HexM) capable of hydrolyzing GM2. A self-complementary (sc) AAV genome was designed with a synthetic promoter to allow packaging of *HEXM*. To test the efficacy of *HEXM* compared to that of the unmodified *HEXA*, these were packaged into scAAV9 vectors and injected stereotaxically into 4 or 15 month old TSD mice along with an identical titer of scAAV9/GFP vector to track vector spread. The mice were euthanized after 4 weeks and brain sections were subjected to IHC analysis against GFP and GM2. The HexA-like activity was assessed by clearance of GM2 within the injected region, compared to the contralateral brain hemisphere. Qualitatively, a marked reduction of GM2 was apparent in the areas of highest GFP expression. The various Hex vectors showed a clear difference in their ability to degrade GM2. As predicted, human *HEXM* was more capable of clearing GM2 aggregates than human *HEXA* at either 4 or 15 months of age. Interestingly, mouse *HEXA* was more effective than human *HEXA*, indicating a species-incompatibility, presumably in the formation of a human α - and murine β -subunit heterodimer. This incompatibility further reinforces the utility of HexM to form a functional homodimer independent of the endogenous α - and β -subunit, which should be effective in treating both TSD and SD.

In conclusion, modified *HEXM* can form a functional homodimer capable of clearing GM2 aggregates. It can be packaged in a scAAV9 vector, which is amenable for strategies directed at widespread CNS gene transfer. These technological advances overcome previous barriers and provide pivotal reagents to develop a translatable gene therapy for TSD and SD.

Keywords: AAV Vectors; Neurological Disorders; Genetic Diseases

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