

# A Direct Gene Transfer Strategy via Brain Internal Capsule Reverses the Biochemical Defect in Tay-Sachs Diseases (2005)

In the early 2000s, Sabata Martino and a team of researchers in Italy and Germany showed that they could reduce the symptoms of Tay-Sachs in afflicted mice by injecting them with a virus that infected their cells with a gene they lacked. Tay-Sachs disease is a fatal degenerative disorder that occurs in infants and causes rapid motor and mental impairment, leading to death at the ages of three to five. In gene therapy, researchers insert normal genes into cells that have missing or defective genes to correct genetic disorders. The team created a virus that coded for a specific gene missing in mice with Tay-Sachs. That missing gene is called hexosaminidase subunit alpha (HEXA). Martino and the team injected the virus into the brains of mice with Tay-Sachs in attempt to restore Hexa enzymatic function in the brain and spinal cord.

The researchers who carried out the experiment were Sabata Martino, Peggy Marconi, Brunella Tancini, Diego Dolcetta, Maria Gabriella Cusella De Angelis, Pia Montacucci, Gianni Bregola, Konrad Sandhoff, Claudio Bordignon, Carla Emiliani, Roberto Manservigi, and Aldo Orlacchio. The team consisted of biochemists and biologists who worked in several institutions such as the San Raffaele Telethon Institute for Gene Therapy in Milano, Italy, the Kekulé Institute of Organic Chemistry and Biochemistry at the University of Bonn in Bonn, Germany, and the University of Pavia in Pavia, Italy. Prior to the team's Tay-Sachs mice experiment, several studies reported the success of therapeutic treatments done in vivo or in the body to treat Tay-Sachs. Those studies also used viral vectors, which restored enzymatic function in tissues in the liver, kidney, and spleen. Those other therapeutic approaches, however, did not affect the central nervous system.

Martino, the lead researcher, designed the experiment for the treatment of Tay-Sachs in mice based on previous work. Martino studied stem cells and gene therapy at the University of Perugia in Perugia, Italy. Martino previously studied the effect of gene therapy on a disorder similar to Tay-Sachs called Globoid Cell Leukodystrophy (GCL). GCL is a fatal degenerative disease that affects the human nervous system. GCL is caused by diminishing activity of an enzyme called b-galactocerebrosidase. An enzyme is a molecule that accelerates chemical activity in cells. Specific genes in DNA produce enzymes, so when genes mutate, they can no longer produce the enzymes, which can lead to diseases like Tay-Sachs and GCL. Using gene therapy, Martino and a group of researchers attempted to restore the essential functions of that enzyme to slow down symptoms of GCL. When treating enzyme deficiency disorders, like Tay-Sachs, the goal of gene therapy is to restore normal gene function of the gene that codes for that missing enzyme. By restoring all enzymatic function, researchers can slow or decrease symptoms of that disease.

In humans with Tay-Sachs, the gene coding for the enzyme HEXA is missing. Without that gene, the body cannot produce the enzyme hexosaminidase subunit alpha. That enzyme in turn is part of the enzyme b-hexosaminidase A. Once that part joins with a second part, formed from the gene HEXB, the two parts form a functioning enzyme. The enzyme b-hexosaminidase A functions in the brain and spinal cord and is found in lysosomes, structures in cells that break down toxins. Within the lysosome, b-hexosaminidase A helps break down a fatty substance called GM2 gangliosides. Humans with Tay-Sachs do not have that enzyme to break down GM2 gangliosides. As a result, GM2 gangliosides build up in cells to toxic levels and destroy those cells, which are mainly located in the central nervous system. That damage causes the symptoms of Tay-Sachs. The research team hypothesized that if they could prevent the build-up of GM2 gangliosides and restore HEXA

enzymatic function in the central nervous system, then Tay-Sachs symptoms would be slowed.

Similar to Martino's previous research on GCL, in the Tay-Sachs experiment with mice, the researchers used gene therapy to restore missing genes and enzymes in mice with Tay-Sachs. To restore essential functions to the missing gene Hexa in the Tay-Sachs mice, Martino and the team of researchers created a non-replicating herpes simplex viral vector (HSV-1) that specifically had that gene. Viral vectors are viruses that inject their genetic material into human host cells. Those cells then replicate that genetic material, making copies of that virus, which infect other cells. Researchers use some viruses in gene therapy treatments to restore missing genes to human cells. Martino and the research team first removed the genes in the virus that caused the disease. Then the researchers replaced those genes with genes coding for the Hexa enzyme.

The team tested their hypothesis using Tay-Sachs afflicted mice as models for humans with Tay-Sachs. The mice demonstrated a build-up of GM2 ganglioside storage in brain cells caused by a lack of the gene Hexa and its resultant Hexa enzyme, the lacks of which cause Tay-Sachs. The researchers separated the mice into five groups with the mice in groups one, two, and three receiving different amounts of the HSV-1 viral vector, those in group four left untreated, and those in group five unaffected by Tay-Sachs.

The team injected the viral vector into the structure of the brain that controls motor pathways, called the internal capsule. The researchers chose that injection site for two reasons. One of the reasons was because motor skills are one of the main impairments for those with Tay-Sachs. The second reason was because the internal capsule allows for the distribution of Hexa enzymes throughout the central nervous system.

To inject the virus, the researchers anesthetized the mice and placed them on the platform of an injection apparatus. The researchers sliced open the mice skulls and then injected the viral vector into the internal capsule on the left side of the brain. Four weeks after the injection, the researchers killed the mice dissected each mouse's brain. The researchers also analyzed the part of the brain that controls muscular activity, cerebellum, and spinal cord for any restoration of Hexa enzyme activity.

After the researchers sliced and observed each mouse's brain, they reported that the GM2 ganglioside build-up had disappeared in the mice injected with the highest dose of the viral vector. That indicated that the viral vector had produced Hexa enzymes in the mice brains, and that those enzymes had functioned in normal processes of disintegrating the fat buildups. The mice injected with the lower dose of the viral vector showed improvement but not a full disappearance of GM2 gangliosides. From those results, the researchers concluded that the effectiveness of the viral vector depended on dosage. All of the Tay-Sachs afflicted mice showed not only depletion of GM2 gangliosides in both the injected and non-injected hemispheres of their brains but also in their cerebellums. Those results indicated that the enzyme diffused throughout the central nervous system from a single injection site, meaning that the virus spread throughout the cells of the central nervous system and restored the gene Hexa into them. The symptoms of Tay-Sachs in those affected mice had been slowed down. The infected Tay-Sachs mice in the experiment confirmed the researchers' hypothesis that the use of gene therapy via HSV-1 viral vector into the internal capsule would decrease GM2 gangliosides build up and restore Hexa , slowing down Tay-Sachs symptoms.

With their experiment, Martino and the research team designed one of the first successful viral vectors able to diffuse throughout the central nervous system.

## Sources

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