- **Dravet Syndrome (DS)** is a severe encephalopathy characterized by infantile onset, refractory seizures, associated with intellectual, behavioral and motor alterations, as well as increased risk of sudden death. In most cases (~90%), the genetic basis is haploinsufficiency caused by mutations in the *SCN1A* gene, which encodes a voltage-dependent Na+ channel type 1 (**Nav1.1**).
- Current anti-epileptic drugs have limited efficacy in DS, despite aggressive combinatorial therapy.
- Due to the complex physiopathology of DS, **etiological approaches such as gene therapy** have unique chances to obtain a global improvement in the life of these patients.
- **Helper-dependent adenovirus vectors (HD-AdV)** are very promising tools for therapeutic gene delivery, since they present a high cloning capacity (>30 Kb), and combine long-term expression with high transduction efficiency.

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Scope of the Problem	· DS is a rare brain disease with an average incidence of 1:20,000 births. So far there is no curative treatment and the current anti-epileptic drugs present limited efficacy. · The vast majority of DS cases are caused by heterozygous *de novo* mutations in *SCN1A*, which encodes for the Nav1.1 channel, pivotal for neuronal activity. Other mutations, that likely cause partial reduction in NaV1.1 channel function, are frequently associated with additional neuropsychiatric disorders including febrile seizures, genetic epilepsy with febrile seizures plus (GEFS+), autism, intellectual disability, familial hemiplegic migraine and age-related cerebral impairment. · The loss-of-function of Nav1.1 channels alters the sodium currents and action potential firing in GABAergic inhibitory neurons causing a general brain hyperexcitability leading to seizures and invalidating neuropsychiatric manifestations.
HD-Ad vectors	· High cloning capacity (>30 Kb) and genetic stability, which allows delivery of large genes such as *SCN1A* (~6 Kb) and complex regulatory sequences. · Long-term expression with high transduction efficiency in vivo. · Genes carried by HD-Ad vectors have been efficiently transduced in brain.

HD-AdV-SCN1A

- Several HD-AdV-SCN1A vectors, carrying the *SCN1A* gene under the control of different promoters, have been generated and their expression tested *in vitro* and in animal models (C57BL/6 Scn1a$^{wt/mut}$ mice).

Proof of Concept

· *In vitro:* The *SCN1A* cDNA has a propensity to accumulate rearrangements. To circumvent this issue, a codon-optimized cDNA was employed. The optimized version is stable and allows efficient Nav1.1 production (**A, B**). Patch-clamp recordings of single cell demonstrate that the transgenic Nav1.1 is able to change membrane potential. As expected for a Nav channel, tetradotoxin (TTX) blocked the protein, suppressing the change in membrane potential (**C**). Based on these results, a HD-AdV vector harboring a strong constitutive promoter and the *SCN1A* cDNA was constructed, purified, and used to infect neuronal cell lines, resulting in strong production of Nav1.1 (**D**).
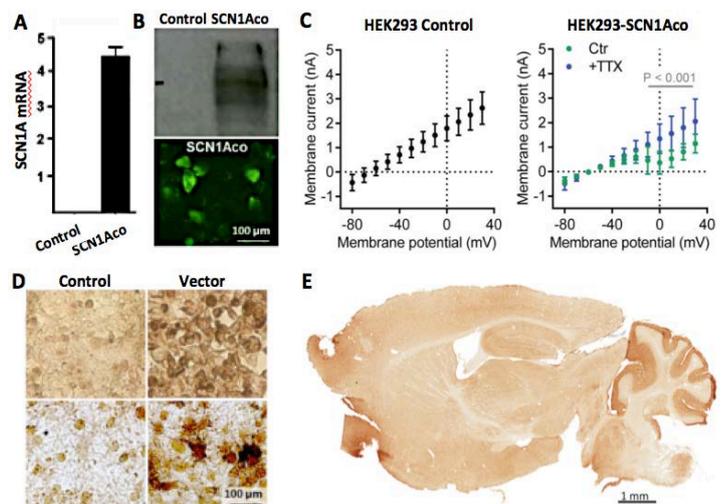
· *In vivo:* Once stereotaxically injected into the brain, the vector induced a significant expression of Nav1.1, which was especially remarkable at the cerebellum (**E**). Treatment was well tolerated, and amelioration of the electroencephalographic signals has been observed. Studies to demonstrate dose-dependent therapeutic effect are ongoing.

Competitive Advantage	HD-AdVs are suitable for gene therapy of DS. Our group counts with the necessary expertise to produce this kind of vectors and to face the challenges imposed by this disease. Moreover, the creation of a robust brain vector will pave the way for GT treatments for other neurological and neurodegenerative diseases.
Intellectual Property	Patent application to be filed.