ANIMAL MODELS FOR GENE THERAPY OF GLYCOGENOSIS

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Since the first human trial in 1990, gene therapy has generated great expectations in society. After over 20 years, there are a lot of gene therapy protocols have reached the clinical stage. In the near future, gene therapy will be an effective alternative to pharmacological efforts, and enable treatment of many diseases that are refractory or not suitable for pharmacologic treatment alone. Thus, gene therapy is a therapeutic tool that gives us virtually unlimited possibilities to develop better and more effective therapies for previously incurable diseases.

Before applying gene therapy in humans it is necessary to do preclinical studies. The aim of these is protect humans of toxic effects that the studied drug may have.

An important element in preclinical studies are animal models. First, tests are made with small animals like mice. If they are successful, then tests are made with larger animals, like dogs. Finally, if these studies give good results then they are passed to higher animals: primates or humans.

OBJECTIVES

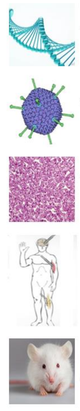
- To gain insight on the role of gene therapy in the field and on the different approaches that are being studied.
- To catch up with the preclinical phases of gene therapy.
- To know glycogen storage diseases and their types.
- To know the assays with animal models in gene therapy.

GENE THERAPY

Gene therapy consists in the transfer of genetic material into cells or tissues to prevent or cure a disease.

There are two types of approaches in gene therapy: in vivo and ex vivo gene therapy (*Figure 1*) [1].

When designing a gene therapy approach there are some key aspects to be considered:



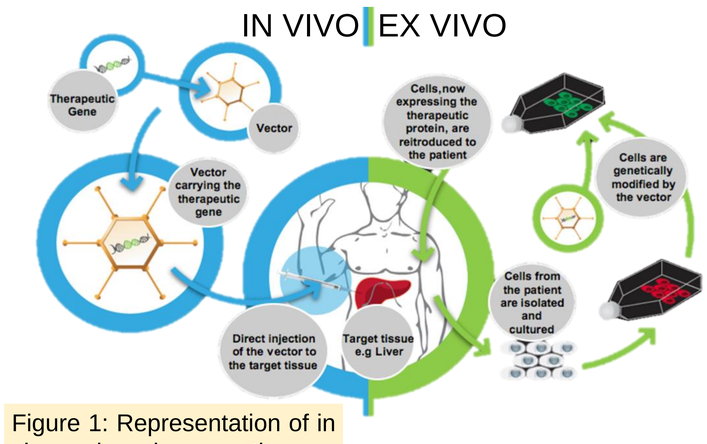
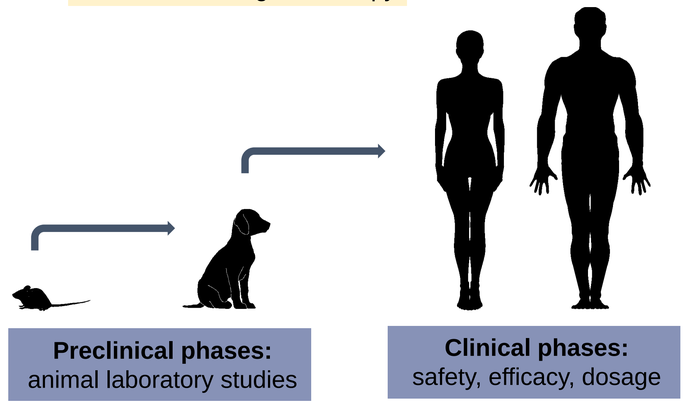


Figure 1: Representation of in vivo and ex vivo gene therapy



GLYCOGEN STORAGE DISEASES (GSD)

Clinical disorder	Gene	Affectation
GSD-0	GYS2	Hepatic
GSD-Ia/Ib (Von Gierke)	G6PC/G6PT1	Hepatic
GSD-II/IIb (Pompe/Danon)	GAA/LAMP2	Multiorgan
GSD-III (Cori-Forbes)	AGL	Multiorgan
GSD-IV (Andersen)	GBE1	Multiorgan
GSD-V (McArdle)	PYGM	Muscle
GSD-VI (Hers)	PYGL	Hepatic
GSD-VII (Tarui)	PFKM	Muscle
GSD-VIII/Ixd	PHKA1	Muscle
GSD-IXa1/IXa2/Ixc	PHKA2/PHKG2	Hepatic
GSD-IXb	PHKB	Multiorgan
Lafora	EPM2A	Multiorgan

Table 1: Types of glycogenosis and the organ affected

GSD are hereditary disorders, usually autosomal recessive, affecting the glycogen metabolism (*Figure 2*). Almost all proteins involved in the synthesis or degradation of glycogen and its regulation have been discovered for some types of GSD.

Depending on the organ that glycogenosis affect we have three types of glycogenosis (*Table 1*).

The frequency of all types of GSD in Europe is about 1/20,000-25,000 live births. The most common, 90% of the total, are: GSD-I, II, III and VI [2].

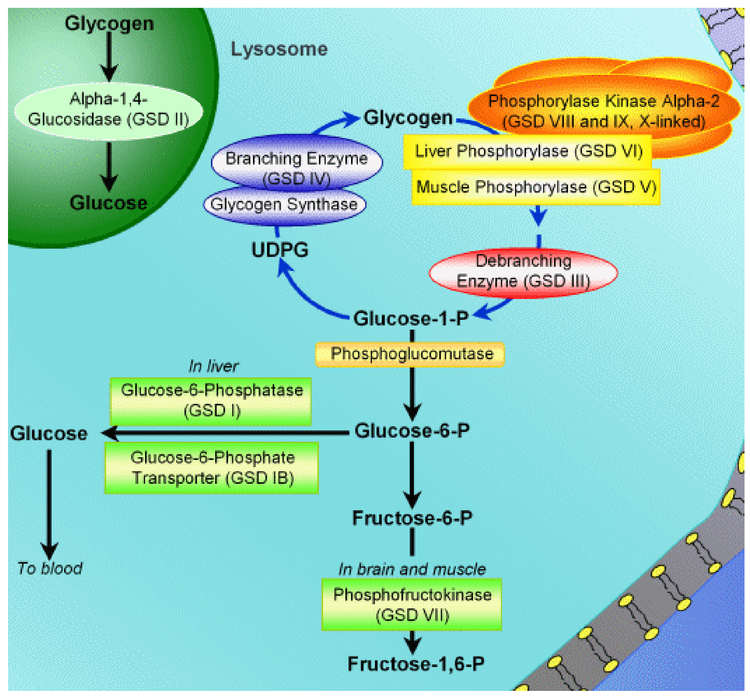


Figure 2: Localization of each type of glycogenosis

HEPATIC GSD: GSD-Ia

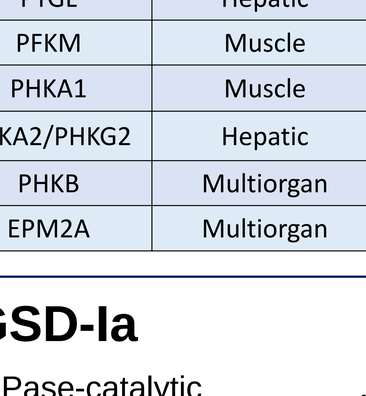
✖ Deficiency in the G6Pase-catalytic unit that hydrolyses G6P into glucose and phosphate in the terminal step of gluconeogenesis and glycogenolysis.

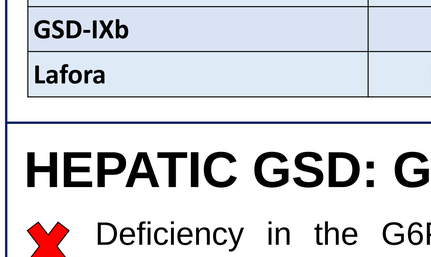
- Hypoglycemia
- Hyperlactacidemia
- Growth retardation
- Hepatomegaly
- Nephromegaly
- Hyperlipidemia
- Hyperuricemia

MOUSE MODEL

Creation a G6Pase-knockout mouse

Introduction Ad-mG6Pase at 2-week-old $G6Pase^{-/-}$ mice





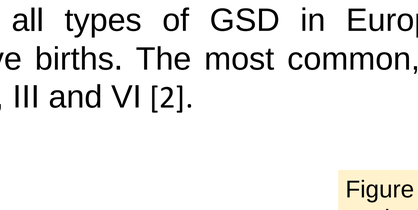
A single administration of Ad-mG6Pase improved the growth rate and completely corrected the metabolic abnormalities in these mice [3].

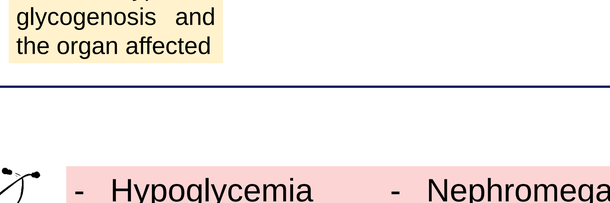
DOG MODEL

Naturally p.M121I G6PC mutation in Maltese Breed

Crossbreed Maltese and Beagle dogs

Administration AAV2/8 vector to affected dog on day 3 or 4 of life





PAS staining revealed decreased liver glycogen (a) 11 weeks following AAV vector administration compared with an untreated (b), affected control liver [4].

SKELETAL MUSCLE GSD: GSD-VII

✖ Mutations in the muscle 6-phosphofructokinase (Pfk).

- Exercise intolerance
- Muscle weakness
- Cramping
- Mild myopathy
- Myoglobinuria
- Compensated hemolysis

MOUSE MODEL

Obtaining transgenic mica without PFK-1 activity

Using the technique of homologous recombination in pluripotent embryonic stem cell and microinjection of blastocysts

$Pfkm^{-/-}$ constitutes a unique model of GSD-VII, which will most likely be very useful for the design and assessment of new therapeutic interventions for this disease [5].

CONCLUSIONS

- Gene therapy represents a promising tool to cure some of those diseases that conventional drug therapies cannot.
- The availability of animal models is key to preclinical phases.
- In GSD-Ia, adenoviral therapy produces only short term corrections and only impacts liver expression of the gene; while AAV-mediated therapy achieves long term correction and the transgene arrives to both the liver and kidney.
- In GSD-VII, the availability of the murine model allows determination of the role of such metabolic alterations in different tissues and organs together with their interactions, and, importantly, allows the study of GSD-VII as a systemic disorder.

REFERENCES

[1] Bosch F, Roca C, Anguela X and Ruzo A. “Gene Therapy, a new tool to cure human diseases” CliniGene-NoE. CBATEG-UAB 2011, http://www.clinigene.eu/video-intró-gene-therapy.html

[2] http://www.glucogenosis.org/portal1/content.asp?ContentID=996#

[3] Chou JY et al (2002) Adenovirus-mediated Gene Therapy in a Mouse Model of Glycogen Storage Disease Type Ia, Eur J Pediatr 161: S56-S61

[4] Beaty RM et al (2002) Delivery of Glucose-6-Phosphatase in a Canine Model for Glycogen Storage Disease, Type Ia, with AAV Vectors, Gene Therapy 9: 1015-1022

[5] Garcia M, Pujol A, Ruzo A, Riu E, Ruberte J et al (2009) Phosphofructo-1-Kinase Deficiency Leads to a Severe Cardiac and Hematological Disorder in Addition to Skeletal Muscle Glycogenosis, PLoS Genet 5(8)