GSD type III: molecular genetics, genotype-phenotype correlations and the project for an animal model

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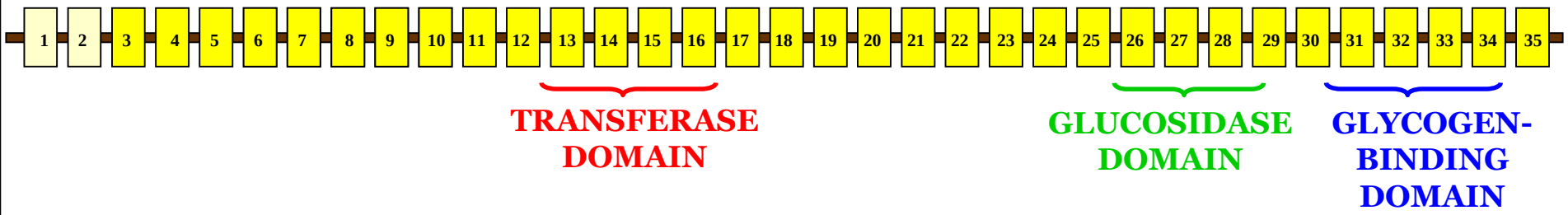
GENE AND DISEASE

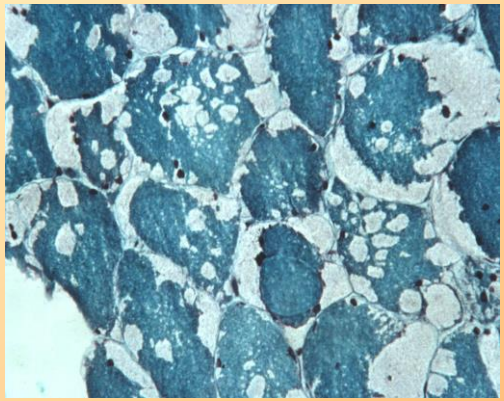
- ❖ AGL gene (amylo-1,6-glucosidase, 4-α-glucantransferase) encodes for the glycogen debranching enzyme.
- ❖ AGL is expressed as a single protein containing two distinct catalytic activities:
  - 4-α-glucantransferase domain located in the N-terminal half of the protein
  - 1,6-glucosidase domain in the C-terminal part

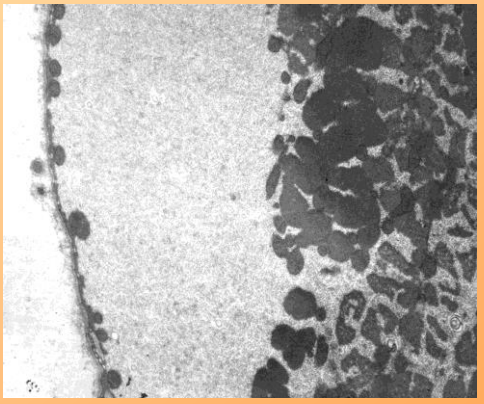


Clinical manifestations

Possible infancy and childhood symptoms:

- Recurrent fasting hypoglycemia
- Seizures
- Hepatomegaly
- Hypotonus
- Growth retardation



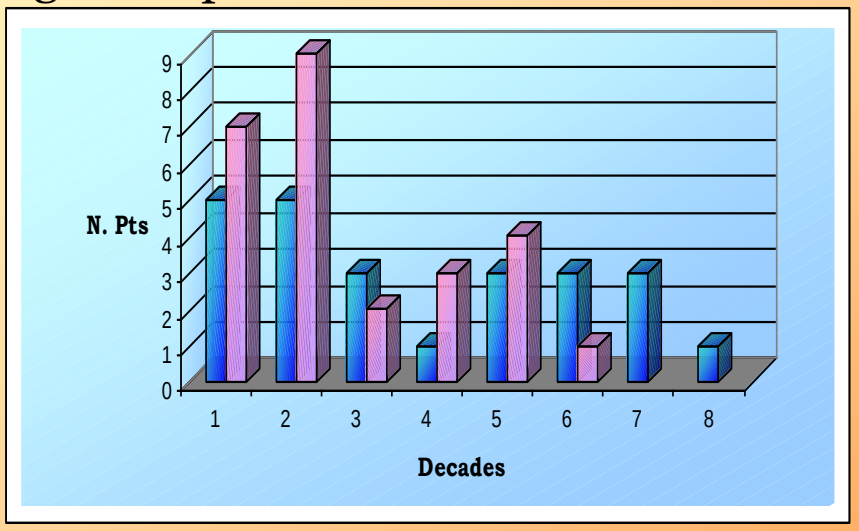


Adult symptoms:

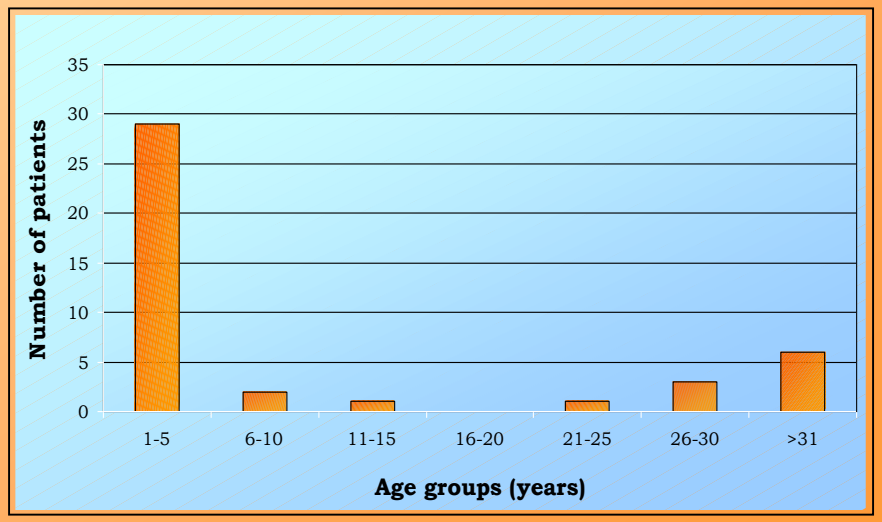
- Distal weakness (calves and peroneal muscles)
- Variable degree of proximal muscles weakness
- Fatigue
- Back pain
- Slow progression
- Serum CK increased 5x to 45x
- Neuropathy (due to glycogen storage in Schwann cells and axons)
- Hepatic dysfunction and cardiomyopathy

FOCUS ON ITALIAN COHORT OF GSDIII PATIENTS

Age Groups

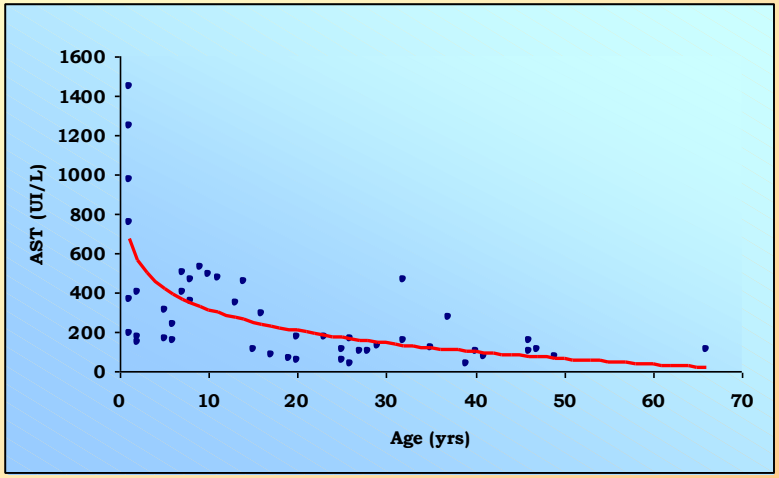


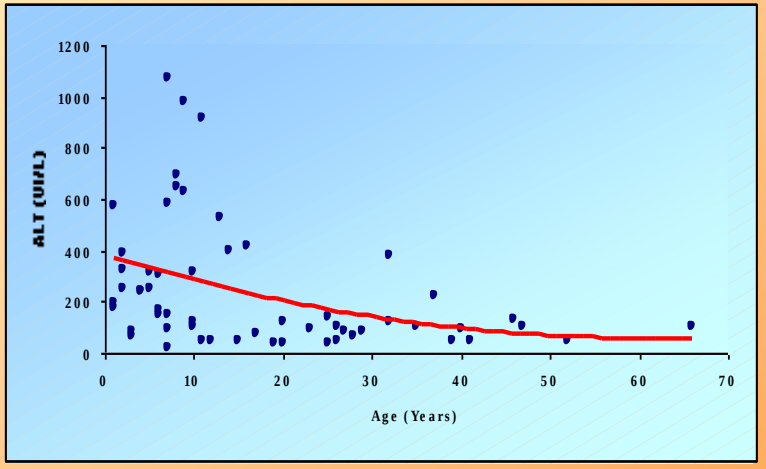
Age at Diagnosis

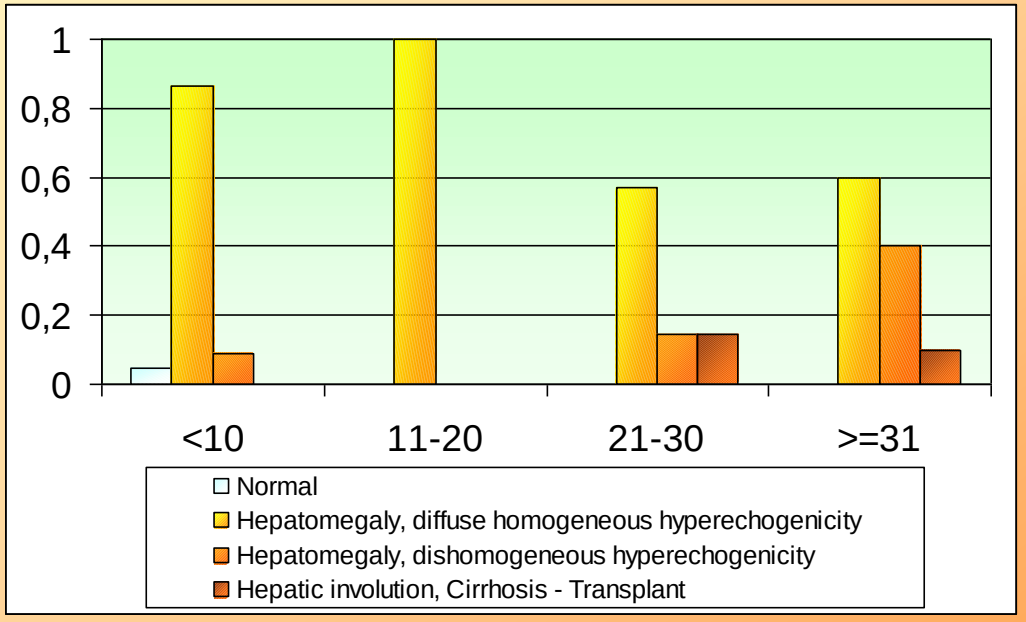


Liver involvement

Serum transaminases



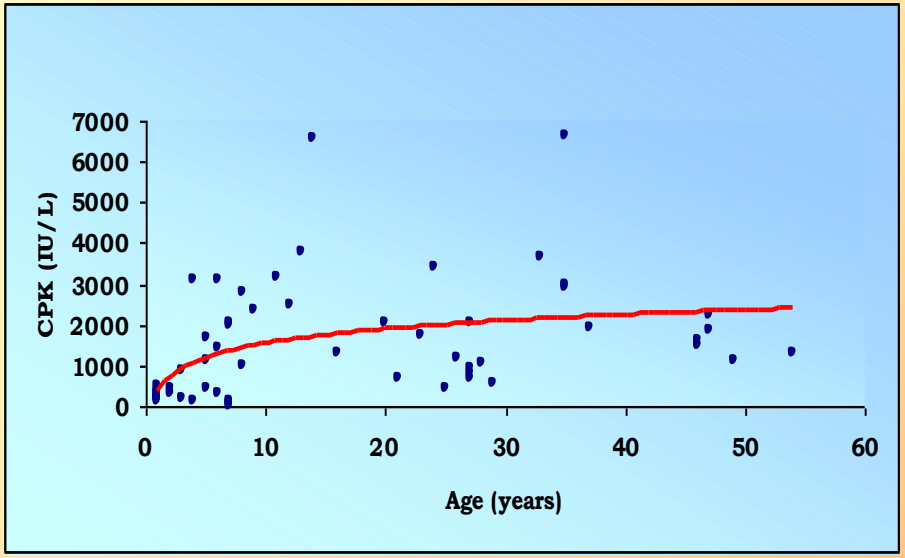




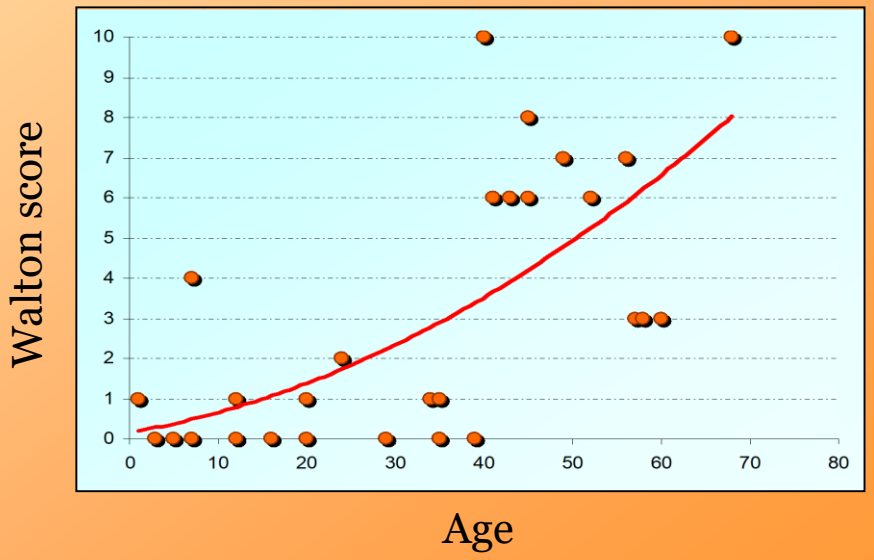
Liver ecography

Muscle involvement

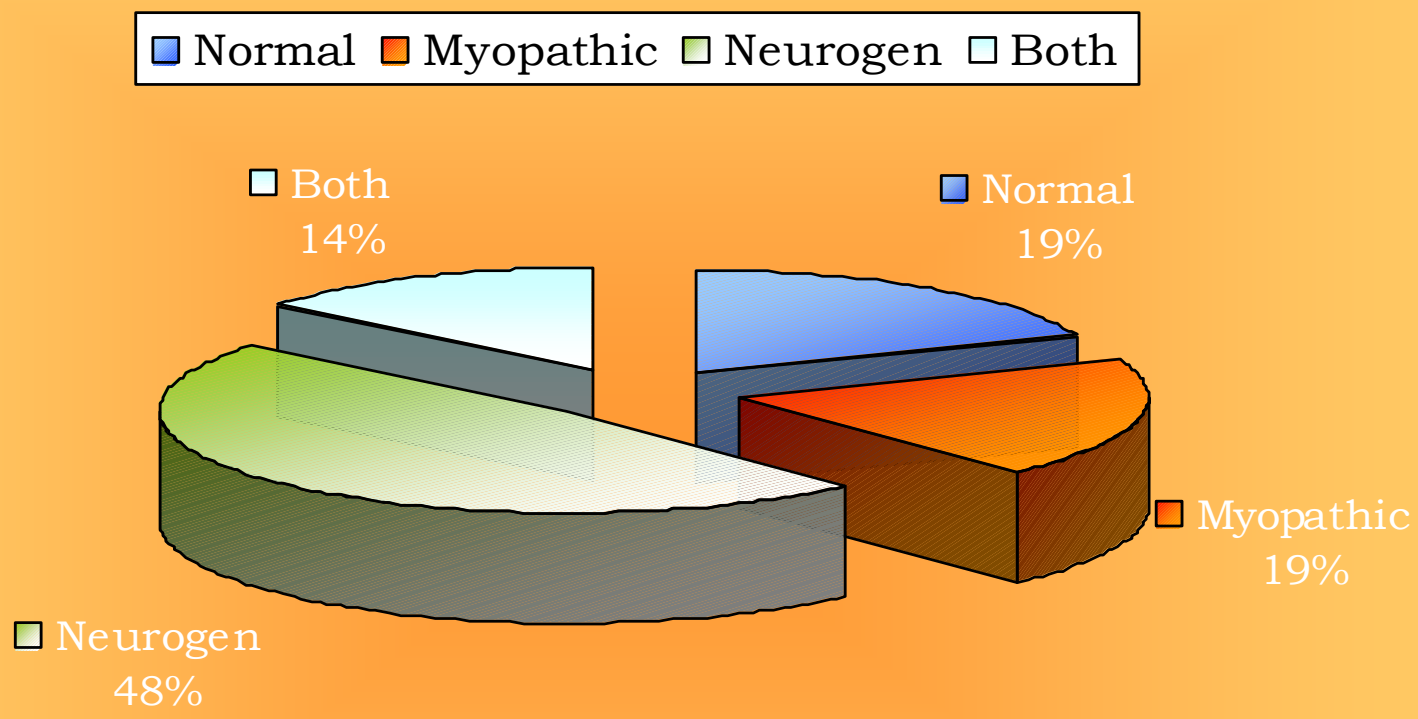
Correlation between CK and age



Walton scale



EMG Findings



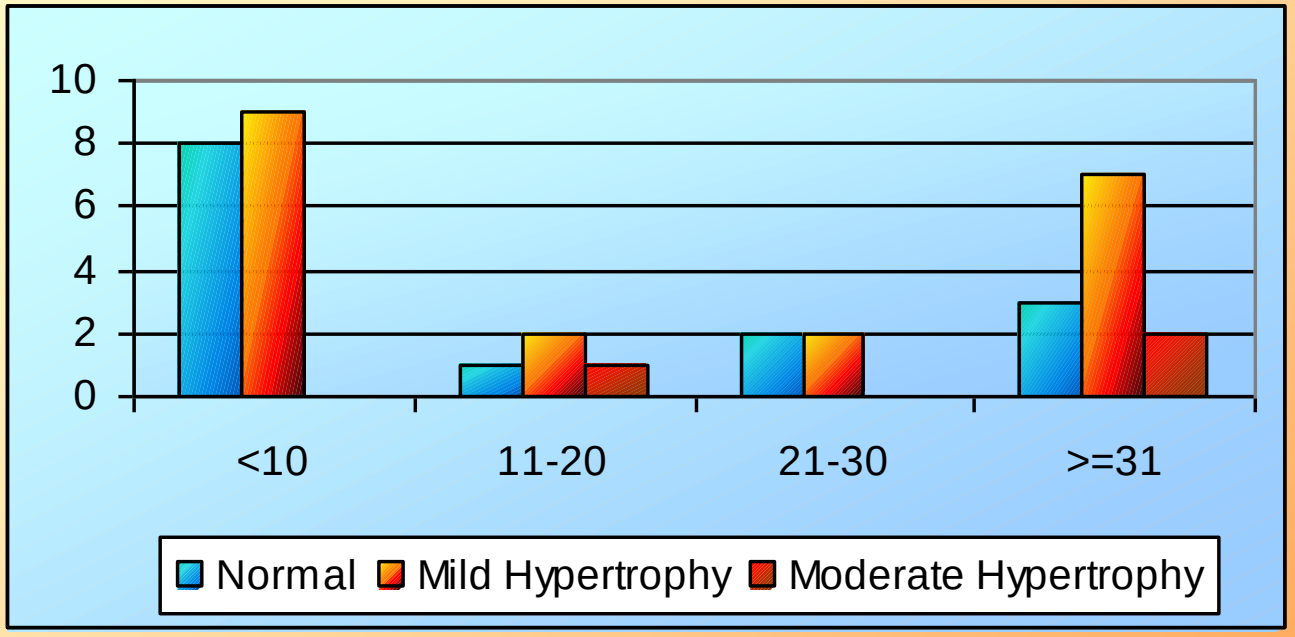
Electromyography was available for 21 patients

Pseudo-myotonic discharges at EMG were found in two patients with myopathic findings and in five showing chronic neurogenic denervation

Normal	**4**
Myopathic	**4**
Neurogen	**10**
Both	**3**

Cardiology

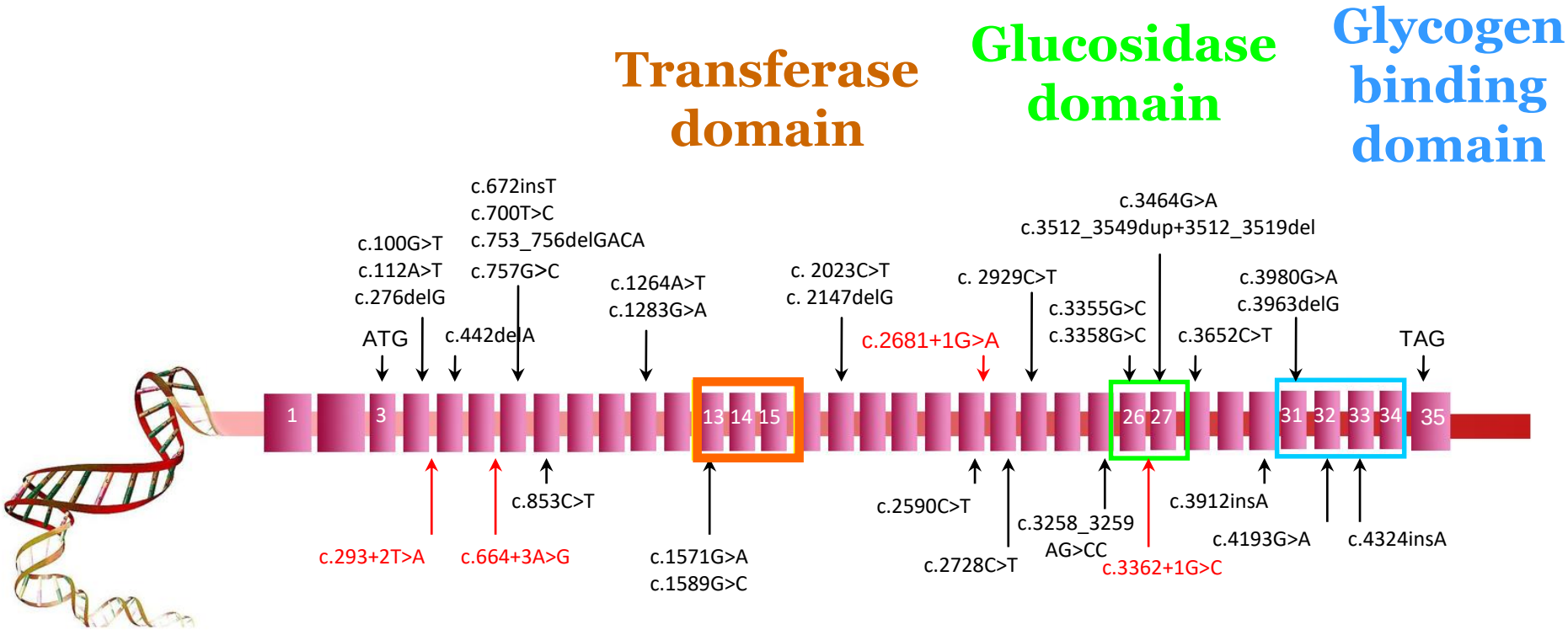
Heart echography



Interventricular sept thickness:

- normal: <10 mm
- mild hypertrophy: 11-12 mm
- moderate hypertrophy: 13-14 mm
- severe hypertrophy: >14 mm

DNA mutational analysis



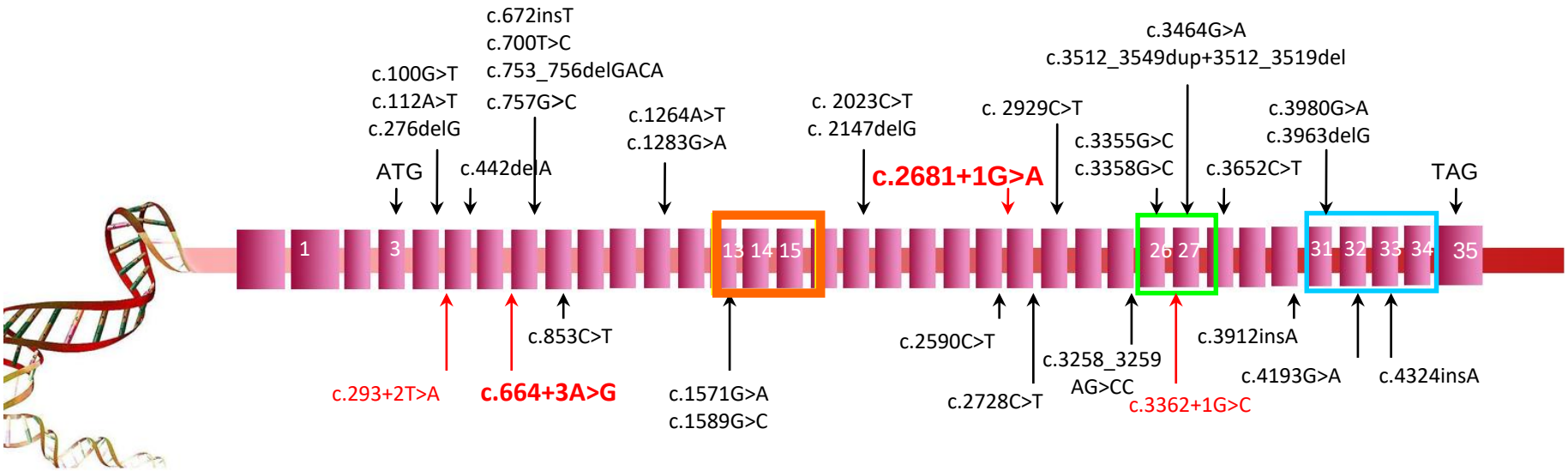
Genetic screening of 57 patients:

- 38 patients (66,7%): 2 mutations
- 7 patients (12,3%): 1 mutation
- 12 patients (21%): no mutations

✓ Mutations are widespread along the whole gene and no hot spot region were found

✓ Private mutations

✓ No mutations were found in exon 3



Mutation analysis:

- 39,8% splicing mutations
- 24,1% nonsense mutations
- 16,9% missense mutations
- 9,6% microinsertions
- 8,4% microdeletions
- 1,2% micro-rearrangements

24,1%: c.2681+1G>A (IVS21 +1 G>A)

10,8%: c.664+3A>G (IVS6 +3 A>G)

Challenging points

1. Molecular screening of the coding sequence and exon-intron junction of AGL gene

Mutations in promoter and intron sequences are missed

BUT

this kind of mutations and missense mutations request

FUNCTIONAL ANALYSIS

In vitro models
(fibroblast and myoblast)

Mutated *AGL* cDNA

Feasible for each missense mutation?

Human Molecular Genetics, 2009, Vol. 18, No. 11 **2045–2052**
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Distinct mutations in the glycogen debranching enzyme found in glycogen storage disease type III lead to impairment in diverse cellular functions

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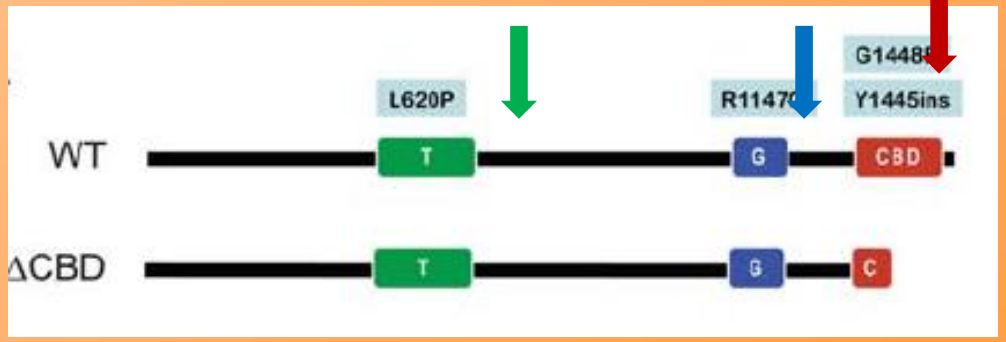
Glycogen binding domain

Glycosidase domain

Trasferasic domain



4 missense mutations in three different functional domains:

- Transferase e glycosidase domain mutations inactivate the specific function of the respective domain and decrease the funtion of the other domain.
- Glycogen binding domain mutations impair both binding and enzymatic activities, proabably through an instability at the protein level.

2. Missense mutations in our cohort account for < 20% of total similar of the findings of other groups working on GSDIII (Goldstein et al., Genet Med 2010)

3. Could it be proper to screen more than 100 **control alleles** as usual?

4. Enzyme activity assay on **red blood cells** does not discriminate among the different catalytic functions but avoid patients to undergo liver or muscle **biopsy**.

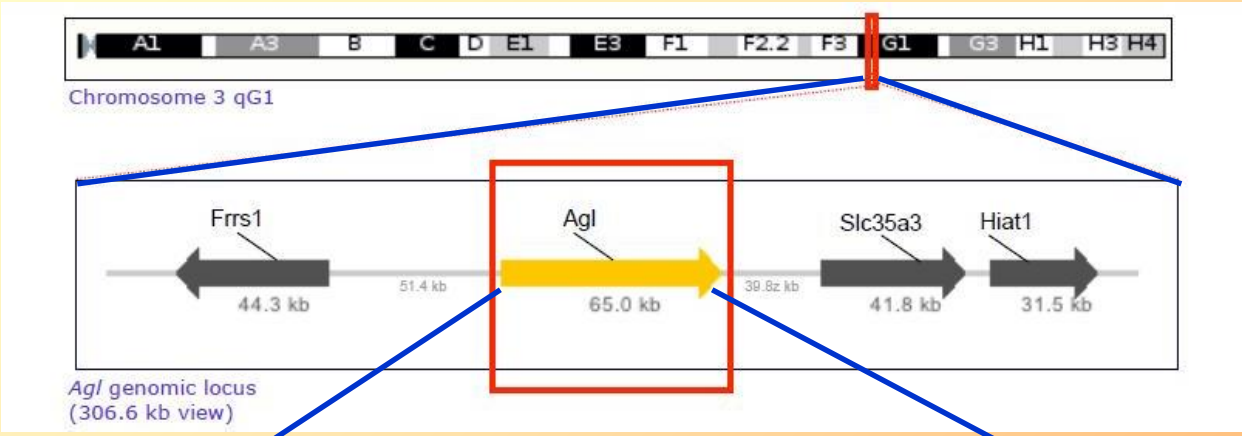
5. Genetically undiagnosed patients often lack enzyme activity assay which should come as a first step. Are those patients true GSDIII?

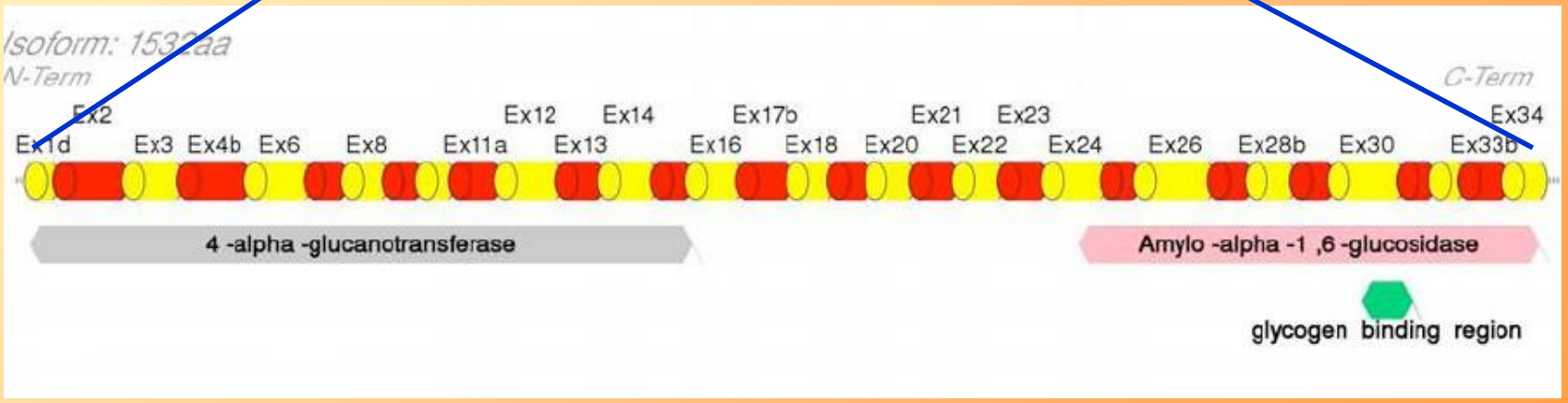
MOUSE MODEL

Associazione Italiana Glicogenosi

www.genoway.com

MOUSE *Agl*: locus and gene organization





Different hypothesis for the knock-out model

1. Constitutive knock-out by **deletion of exon 1** containing ATG (corresponding to human exon 3)

   The removal of the first ATG should abolish the mRNA translation

2. Constitutive knock-out by **deletion of a functional domain**

The removal of a catalitic domain would not allow the function of the enzyme

3. **Tissue-specific** or **adult-specific** "Safe knock-out™" mouse (targeting exon 1)

This construct would allow the regular expression of the enzyme until the induction of the Knock-out, which could be tissue specific or time specific, by breeding the chimera with Cre-recombinant strain

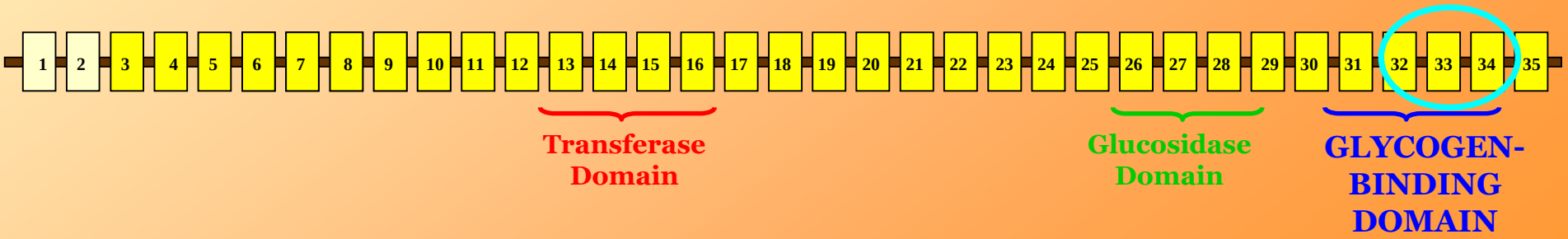
Criteria for the choice of the best mouse model

- We need a model reproducing the human disease
- AGL is expressed in many tissues
- Mutations do not seem to affect fertility and fetal development

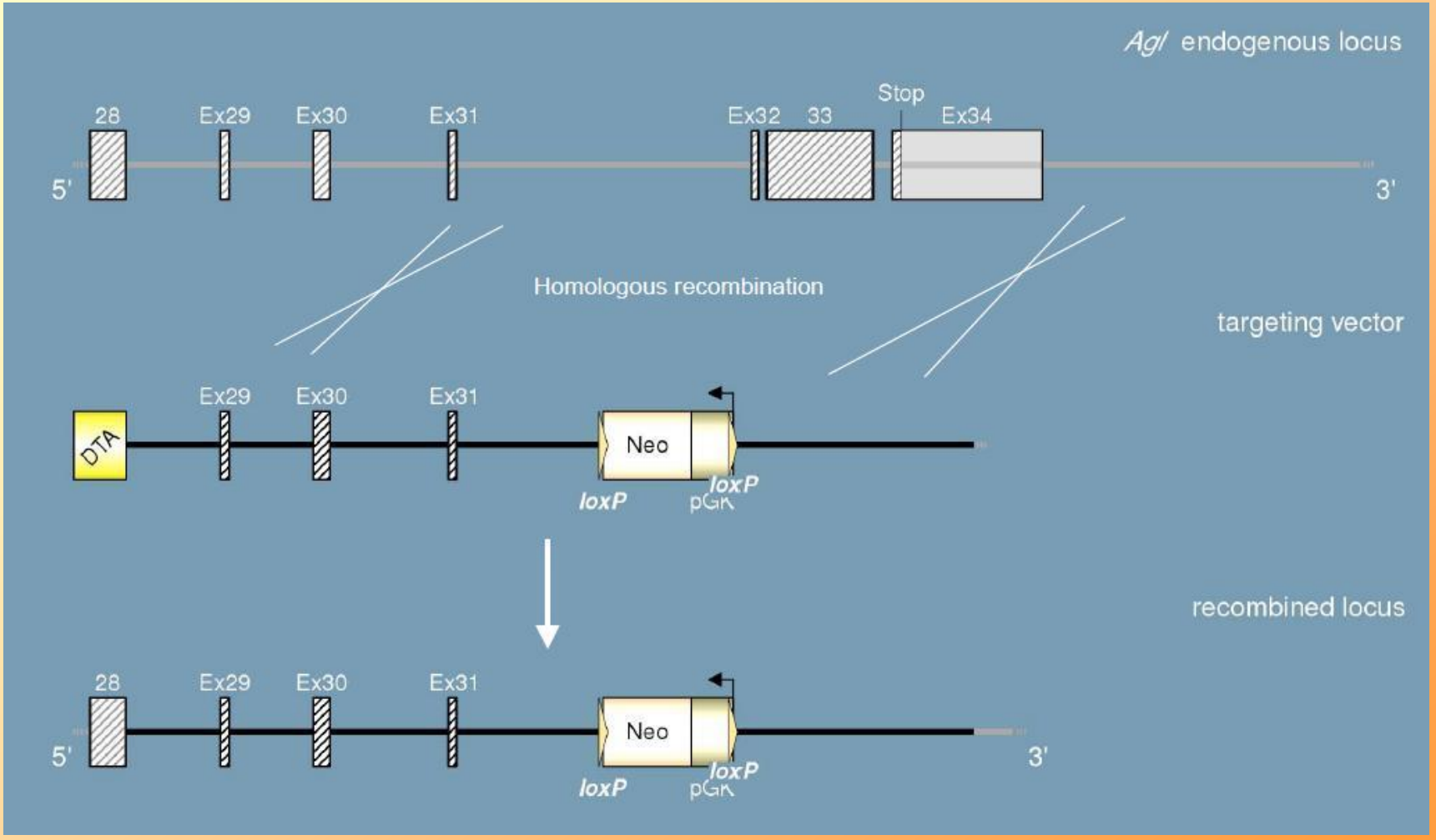
→ **Constitutive knock-out model**

Evidences that deletions of the C-terminal of the protein abolish both enzymatic activities (Cheng et al., Genes Dev 2007; Cheng et al., Hum Mol Genet 2009)

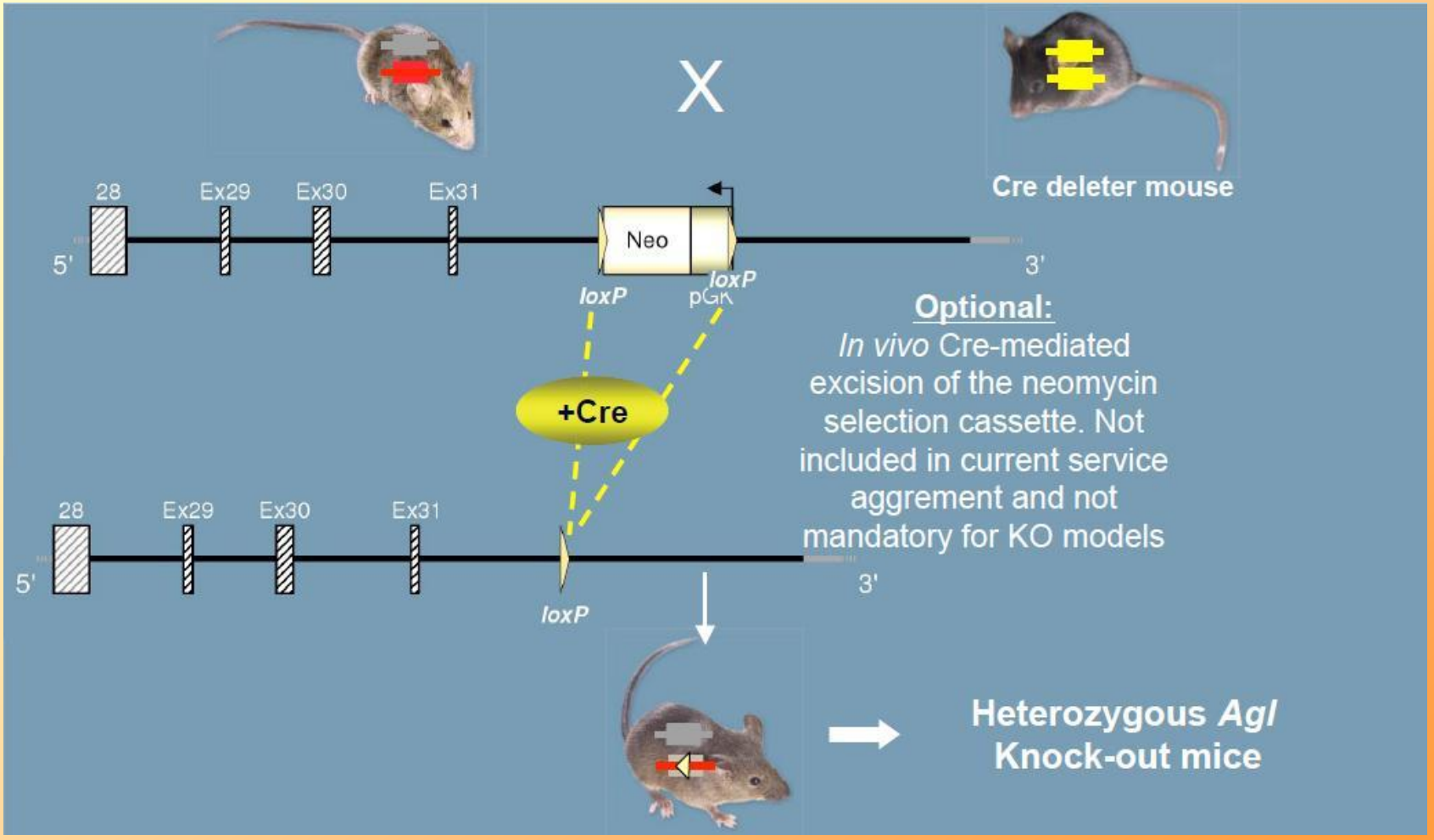
→ **Deletion of the last 114aa of the glycogen-binding domain (ex 32-34)**



Constitutive knock-out model by deletion of the glycogen-binding domain: targeting vector



Constitutive knock-out model by deletion of the glycogen-binding domain



Testing mouse to better understand

- Diet treatment: composition and relation with age
- Physical exercise and metabolism
- Peripheral nerve involvement
- Glycogen deposition vs musculoskeletal impairment

...and more

A good mouse model would allow the development of potential **drugs** and **Enzyme Replacement Therapy**

Thanks to

- Patients
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