

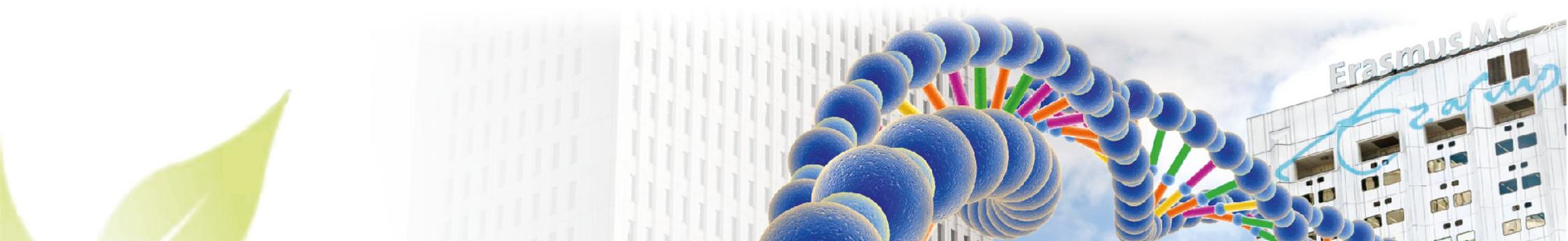
Development of a new treatment strategy for Pompe patients that carry the IVS1 variant

Antisense oligonucleotides for the treatment of Pompe disease

Center for Lysosomal and Metabolic Diseases
Erasmus MC, Rotterdam, Netherlands

AEEG, September 23, 2017

Atze Bergsma, PhD



Hemos hecho mucho, queda mucho por hacer
We have done a lot, a lot more can be done

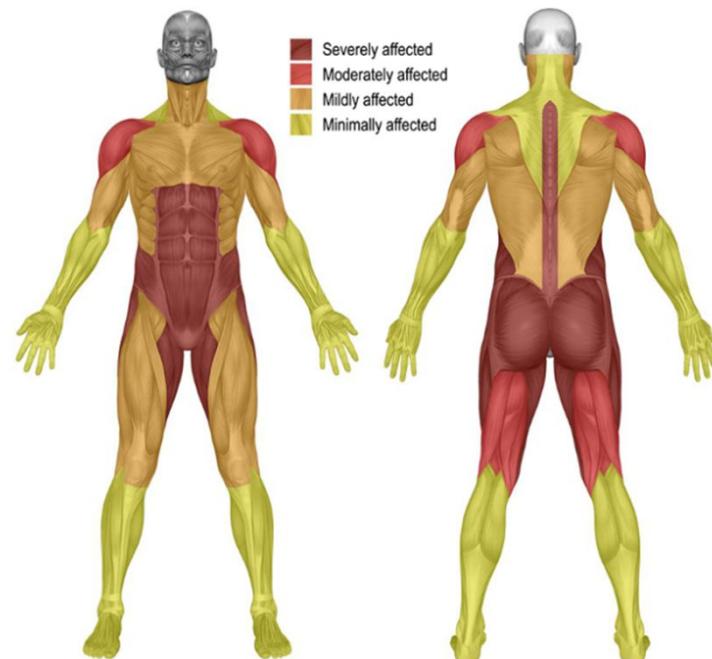
Erasmus Medical Center, Rotterdam, Netherlands



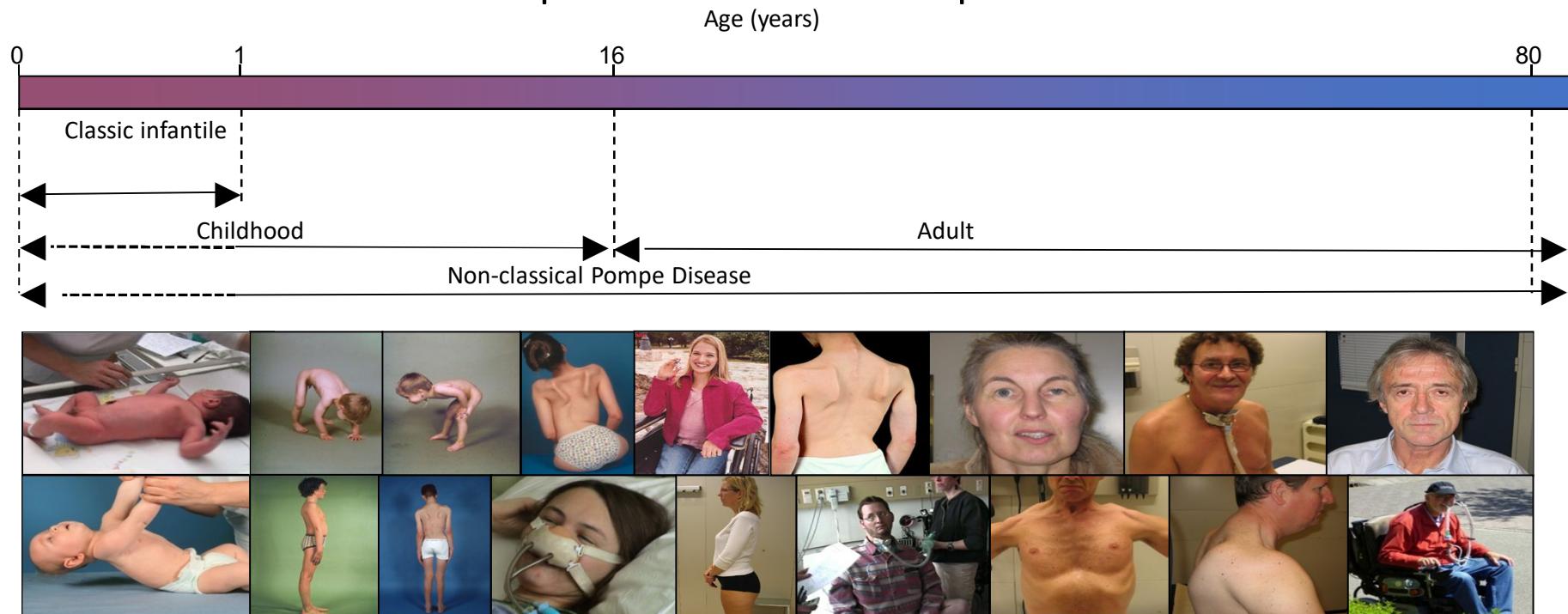
Center for lysosomal and Metabolic Diseases

Pompe disease

- Genetic disease in which one gene is effected
- Acid α -glucosidase (GAA) deficiency
- Lysosomal storage disease
- Accumulation of glycogen in the lysosome
- Mainly affects muscles
- Clinical spectrum of severity



Clinical spectrum of Pompe disease



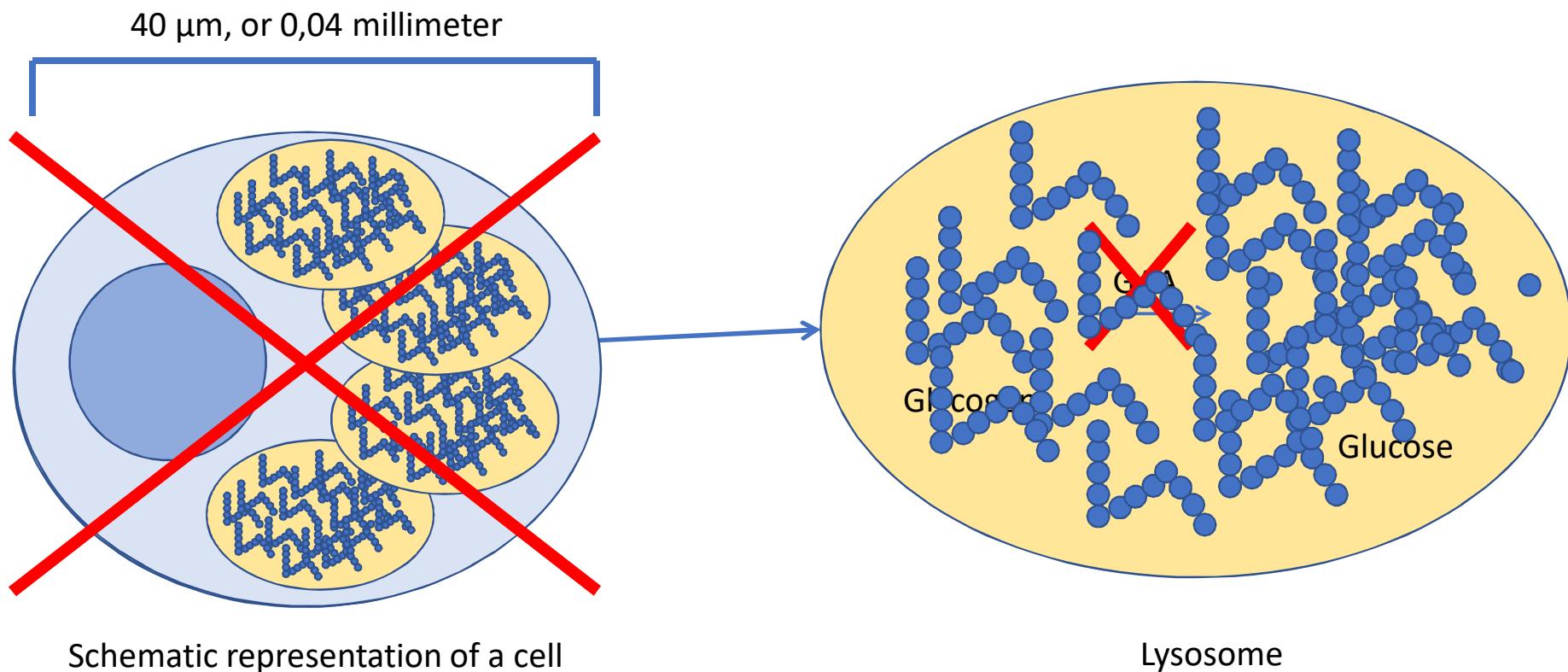
0% → Residual GAA Activity compared to healthy average → 20%



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What causes Pompe disease?

The function of the acid α -glucosidase enzyme

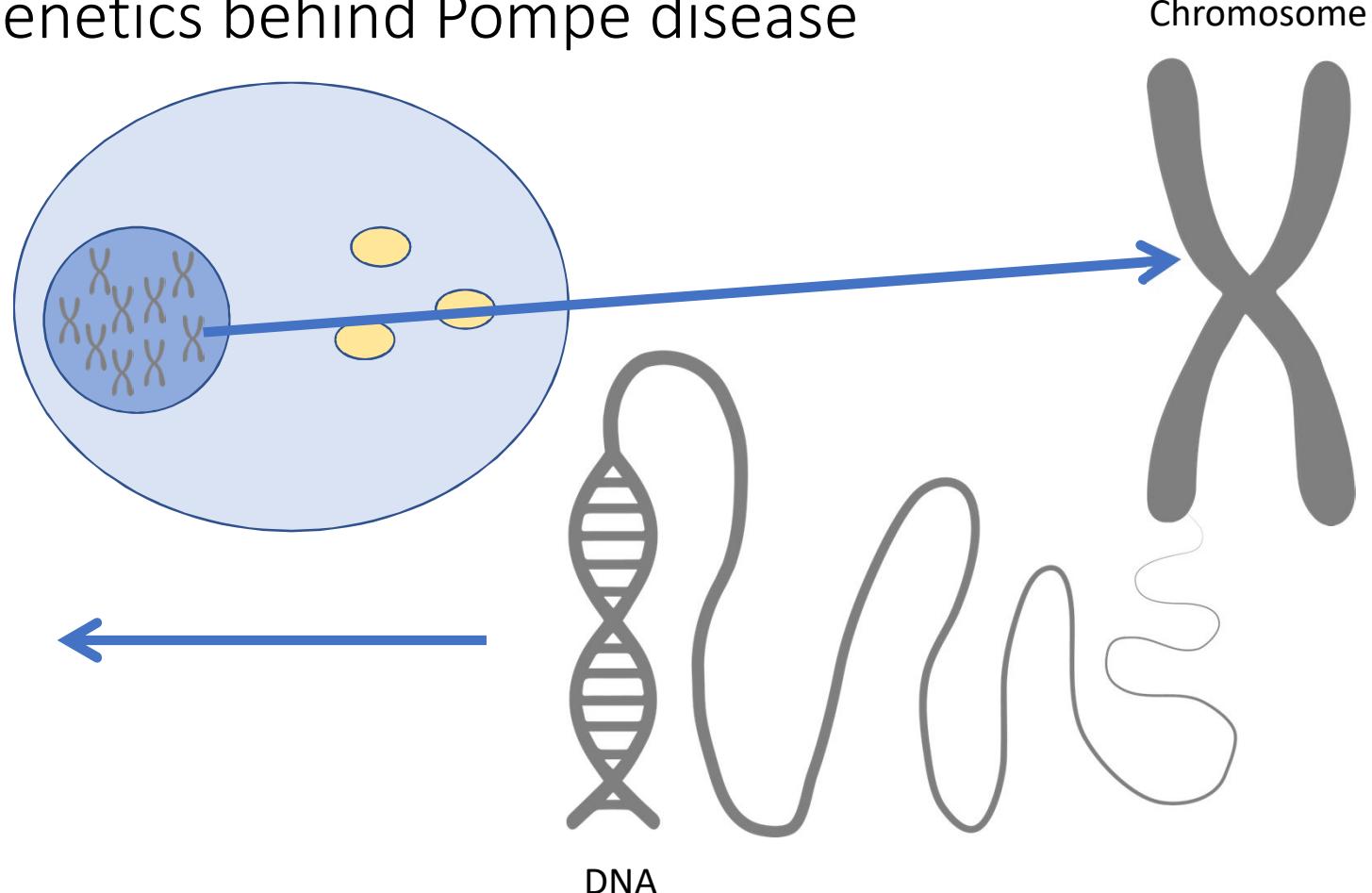
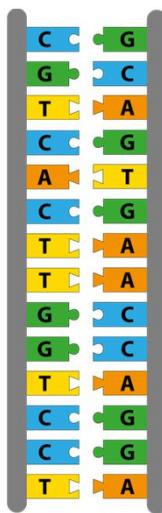


Genetics behind Pompe disease

- Genetics = DNA

DNA
Building Blocks
(bases)

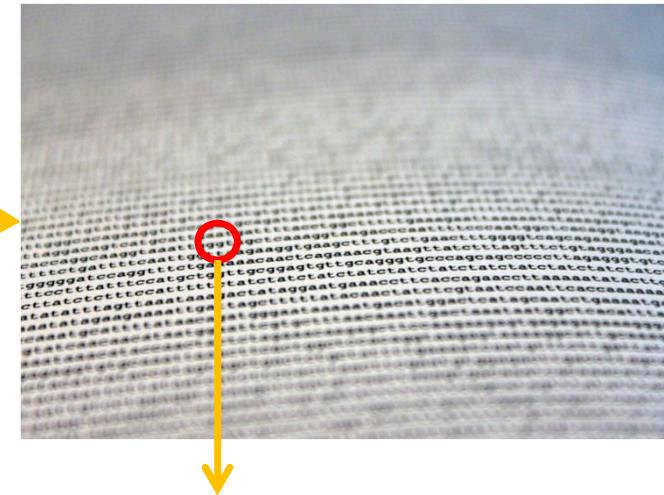
A
T
C
G



Genetics behind Pompe disease

- We have 3.2 billion building blocks in a row in our genome

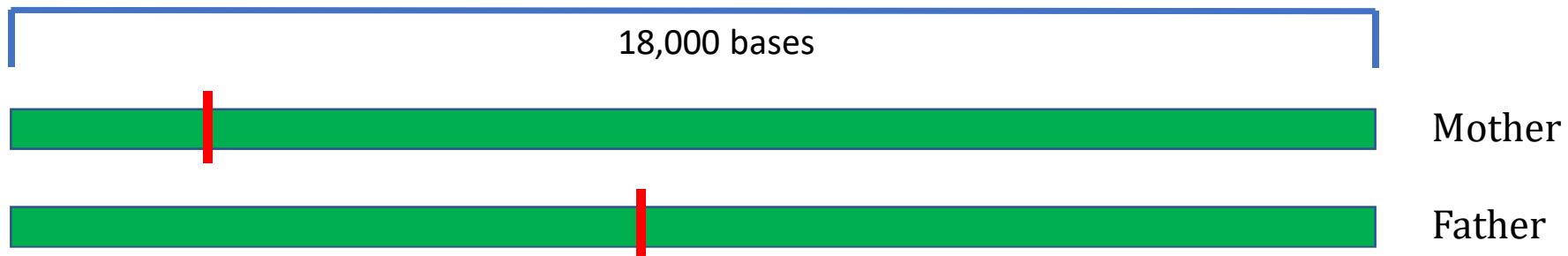
GAA gene = 1 page



Only two changes
(mutations) in the code can
cause Pompe disease!

Genetics behind Pompe disease

Two GAA genes are present in every cell, one from the mother and one from the father:



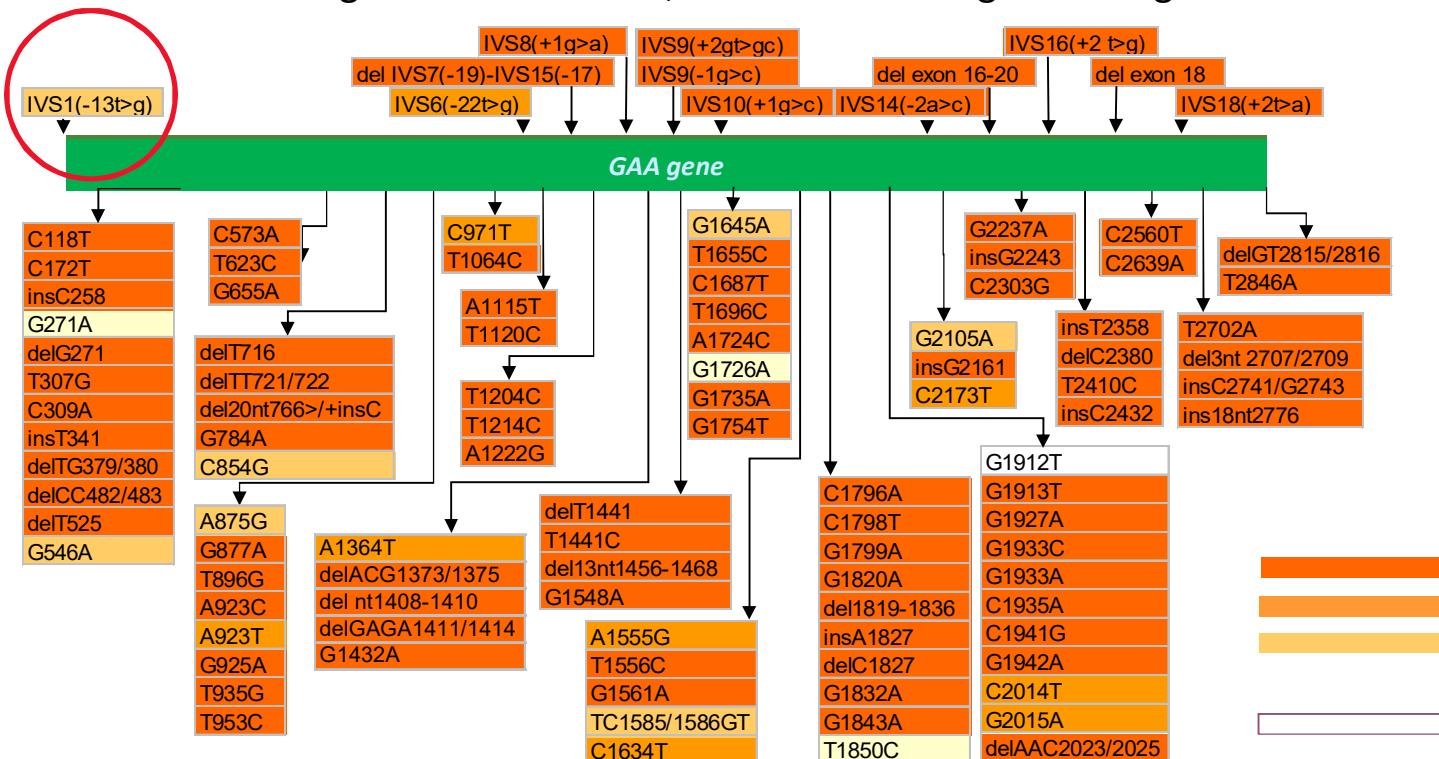
No mutation present -> **100%** of GAA activity -> **Healthy individual**

Mutation in one gene (mother or father) -> **50%-70%** of GAA activity -> **Healthy individual**

Mutation in two gene (mother and father) -> **<20%** of GAA activity -> **Pompe patient**

Genetics behind Pompe disease

Almost 500 mutations in the *GAA* gene are described, distributed throughout the gene.



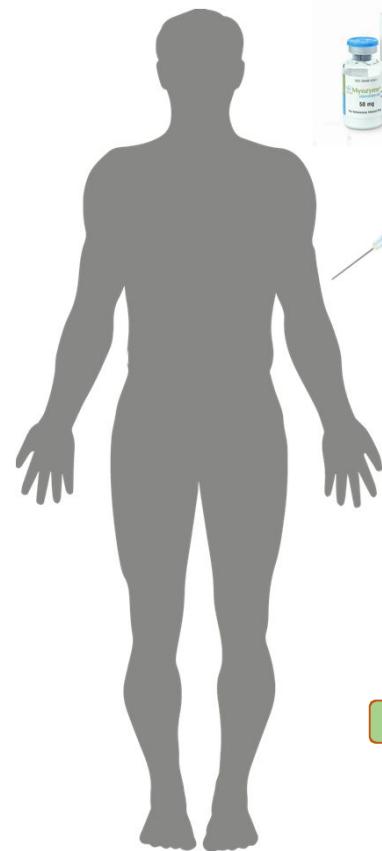
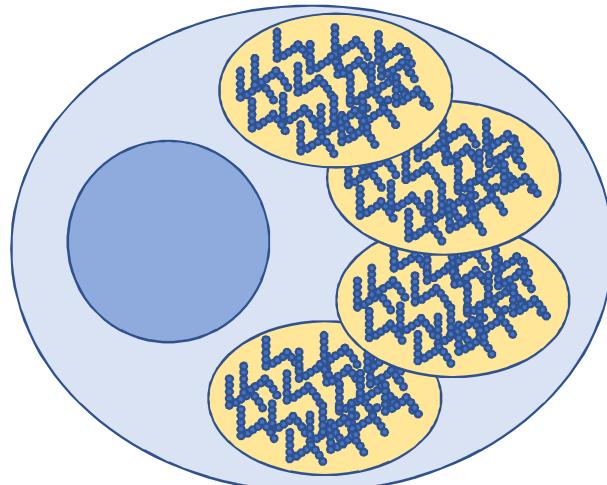
The IVS1 mutation occurs in >70% of Caucasian Pompe patients.

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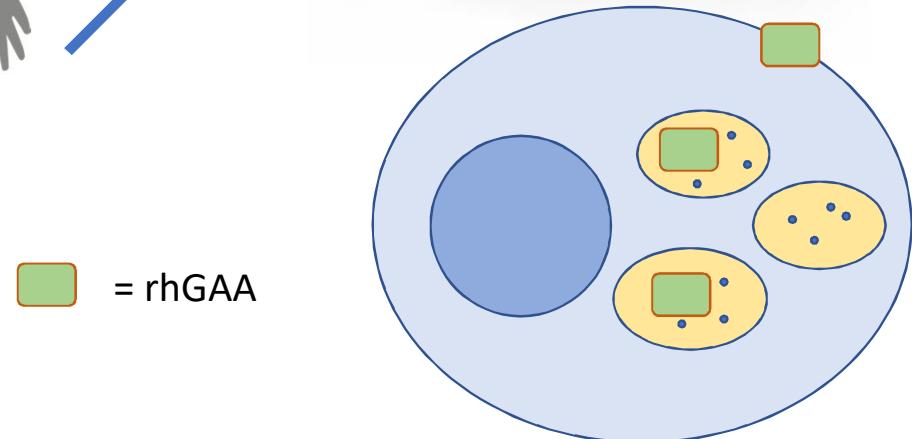
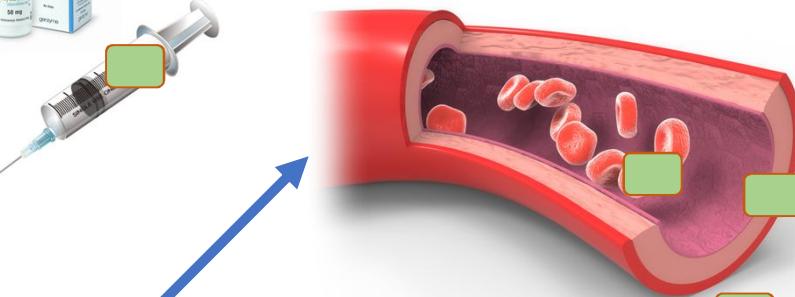
Therapy for Pompe disease patients

Enzyme replacement therapy for Pompe disease

Situation without ERT



Situation with ERT



 = rhGAA

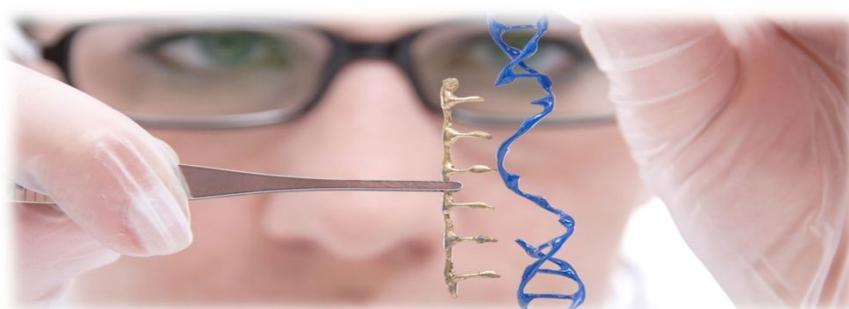
Enzyme replacement therapy for Pompe disease

- Positive effects:
 - Effective in infantile patients.
 - Prolonged life expectancy for adult patients.
- Drawbacks:
 - No full recovery for infantile patients.
 - Low impact on muscle strength for adult patients.
 - Immunological response.
 - Lifelong administration.
 - High costs (≈ 400.000 annually)
- Can we develop another potential drug for treatment of Pompe disease?



Possible therapeutic strategies for Pompe disease

Gene/DNA-based therapy



RNA-based
therapy



Small molecule-based
therapy



More advanced
protein-based therapy



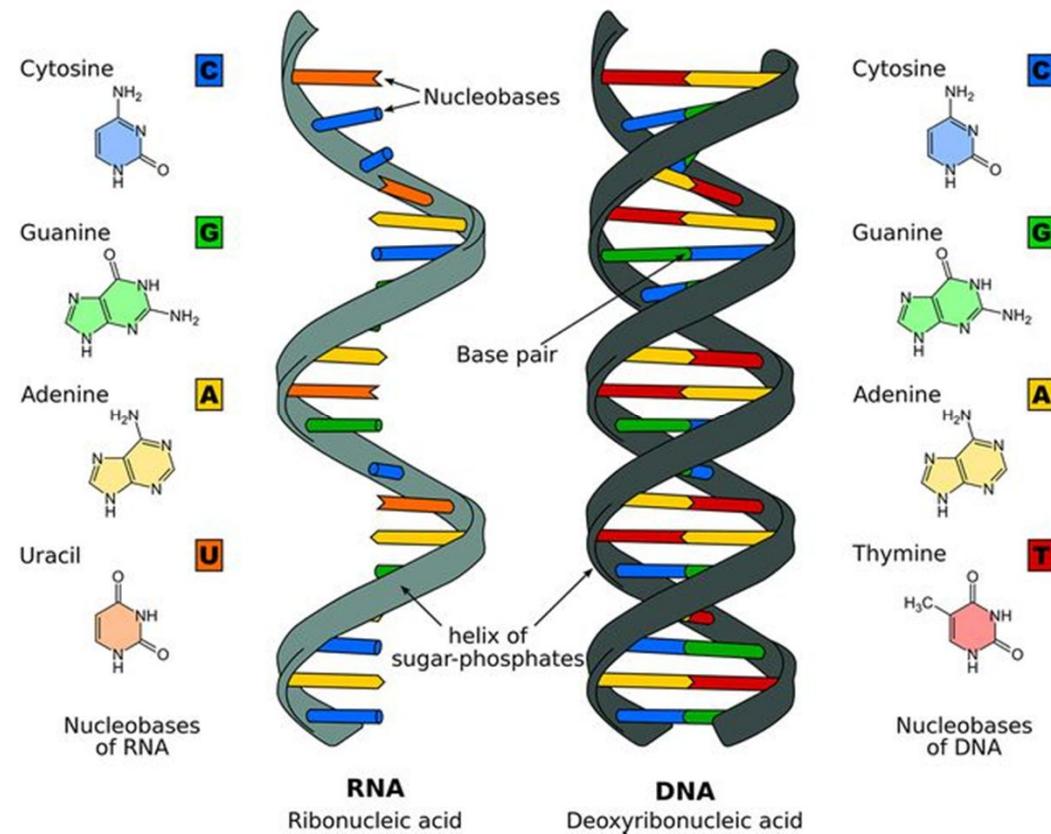
Diet-based therapy



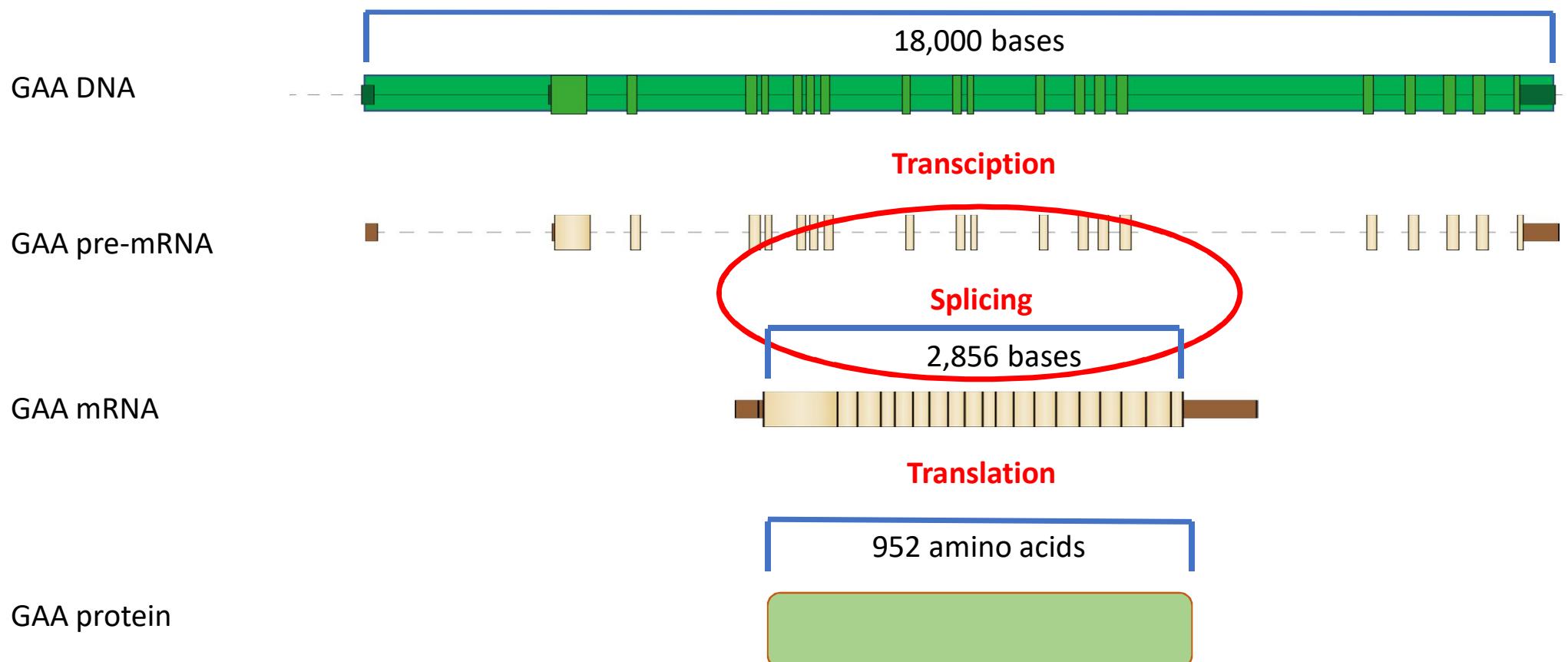
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RNA and Splicing

Difference between RNA and DNA

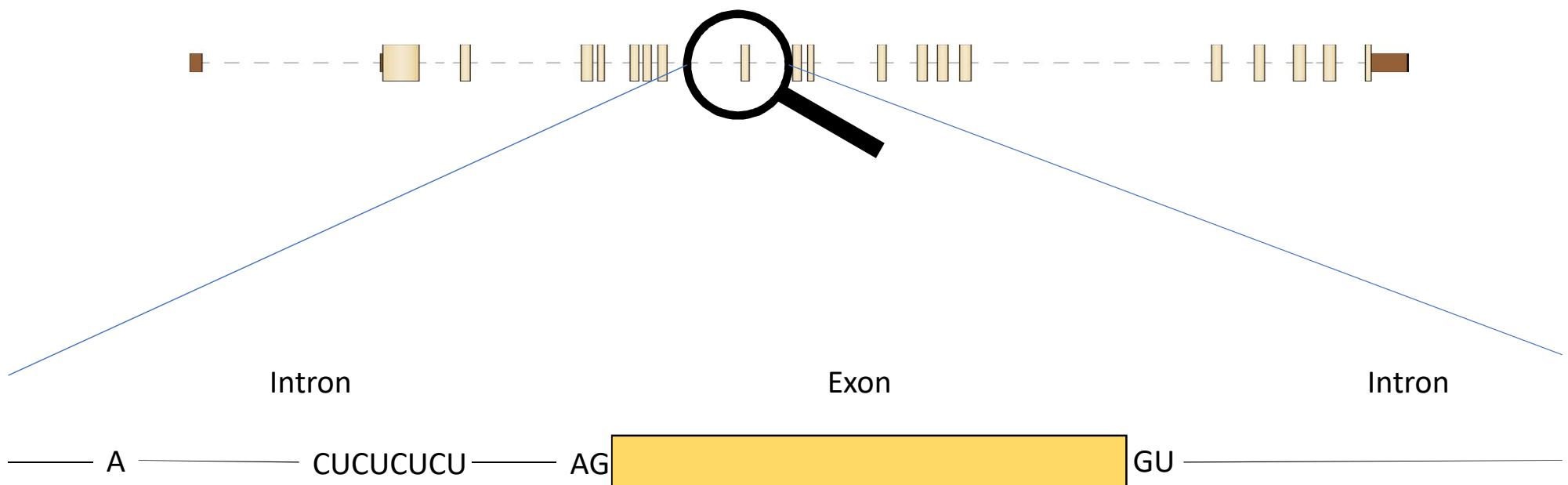


What is RNA?

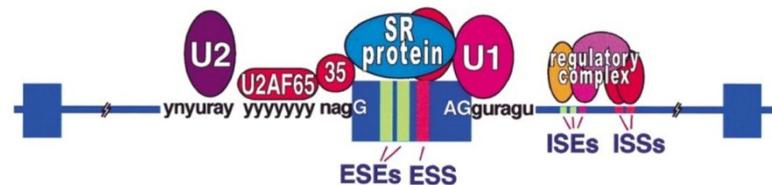
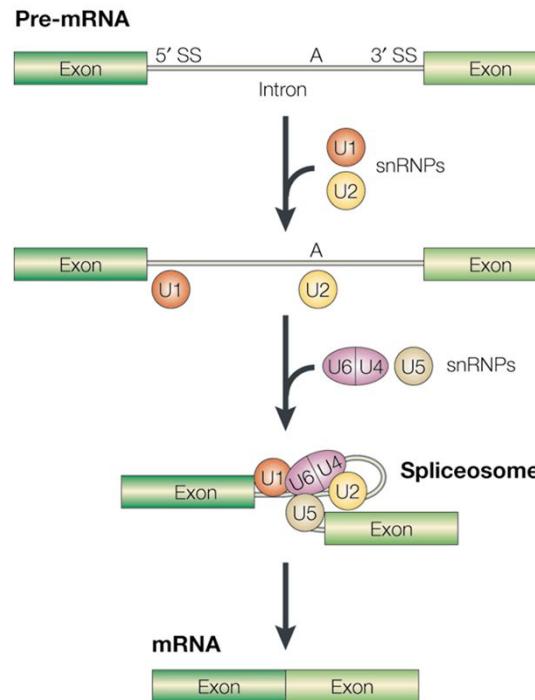


Recognition of cut/paste sites during RNA splicing

Some sequence elements are vital for correct splicing



Regulation of splicing

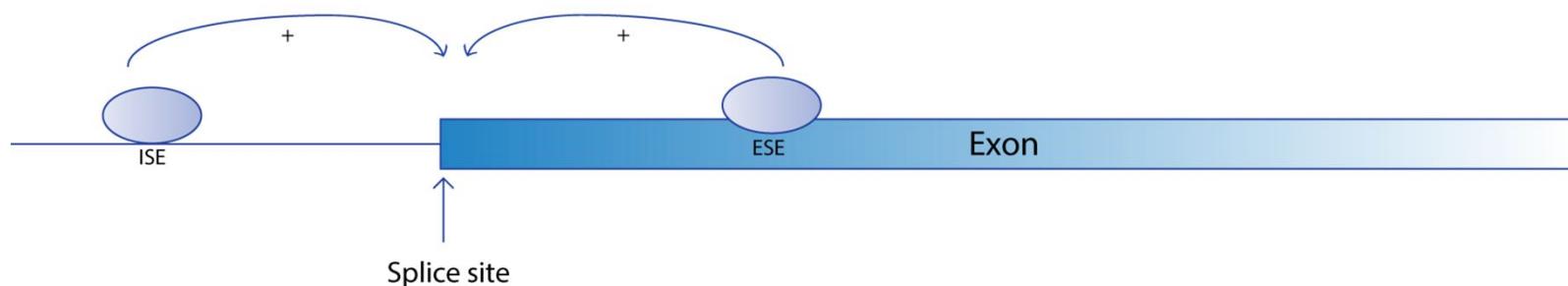


Nature Reviews | Neuroscience

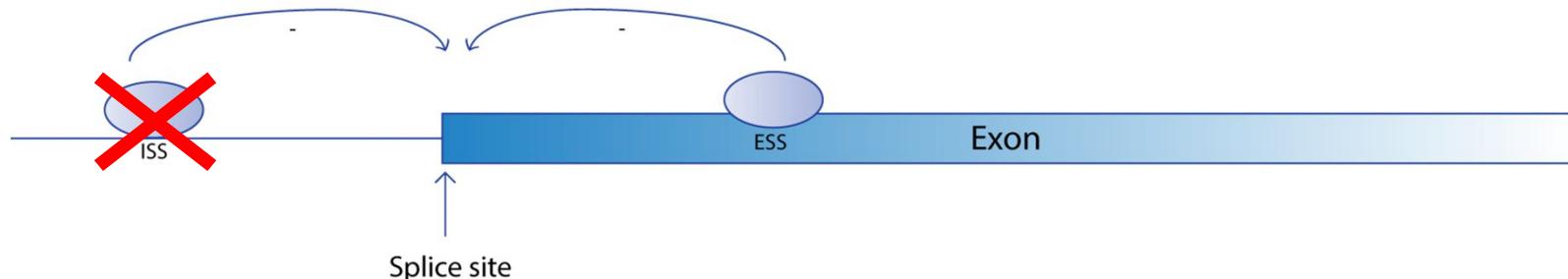
The spliceosome is just the core, but >300 proteins also play a role in splicing

Splicing regulatory elements

- Two important splicing regulatory elements include:
 - Splice Enhancers -> **Positive effect on splice site recognition -> More exon inclusion**



- Splice Silencers -> **Negative effect on splice site recognition -> more exon skipping**



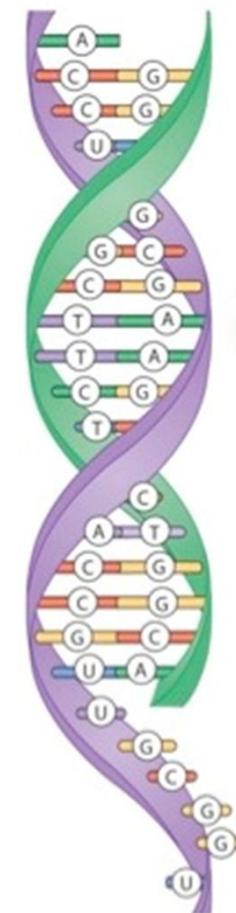
- **Can we block splice silencer elements to improve exon inclusion?**

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Antisense Oligonucleotides (AON)

Antisense oligonucleotides

- AONs are molecules that can bind to RNA in a sequence specific manner
- AONs are small (18-30 bases) RNA mimics
- AONs are stable because of their modified backbone
- Different backbones have different pharmacokinetic properties



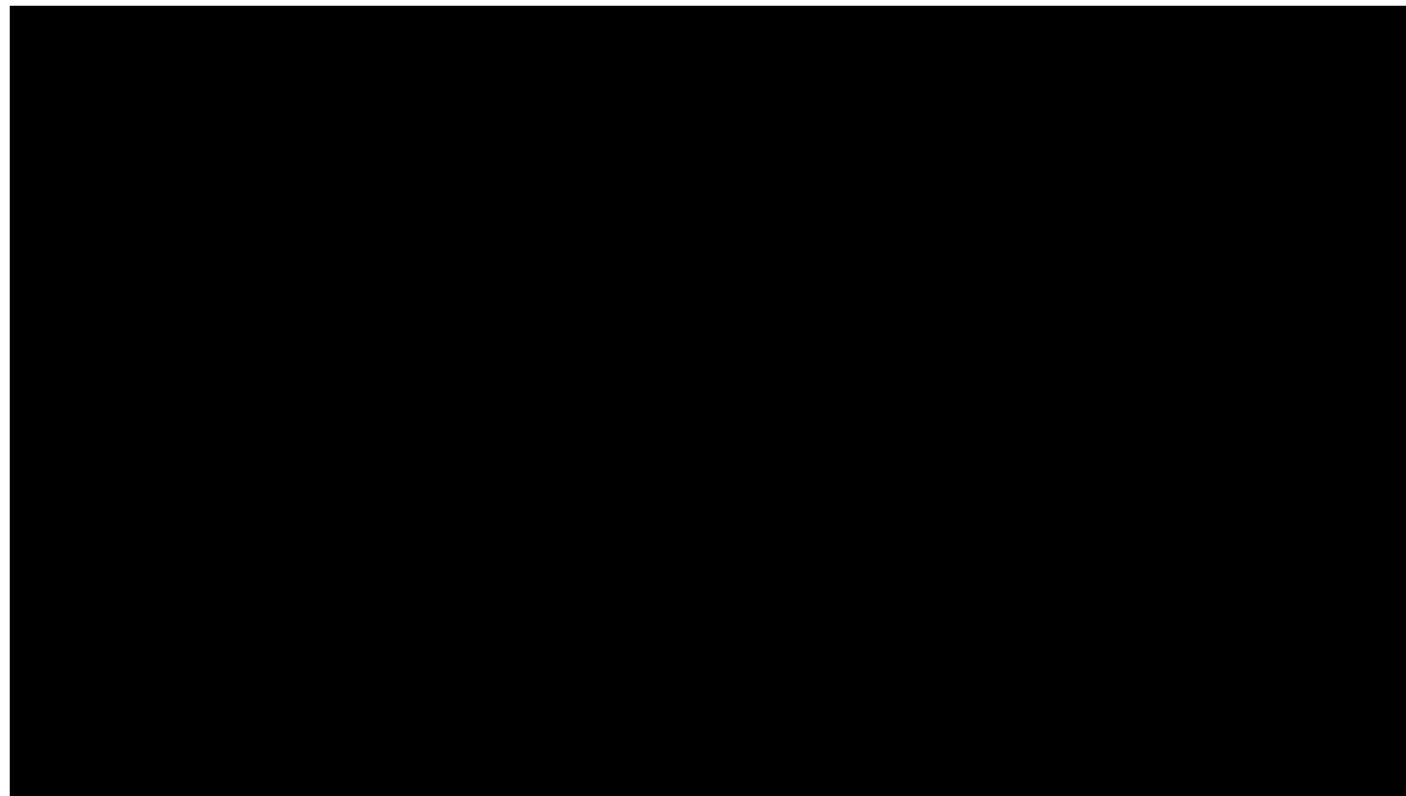
Antisense Therapy: Spinal Muscular Atrophy

- In spinal muscular atrophy the SMN1 gene is defective.
- A second gene, the SMN2 gene, encodes the same protein.
- However, a mutation in SMN2 exon 7 leads to **skipping** of that exon.
- An AON has been designed to block a intronic splicing silencer in intron 7.
- Treatment with this AON leads to exon **inclusion** of exon 7.

5% normal

90% normal

Antisense Therapy: Spinal Muscular Atrophy



Two antisense-based drugs approved by the FDA

Spinraza
Spinal muscular atrophy
(approved Dec. 2016)

**BREAKING!
NEWS!**

FDA has Approved
Spinraza for SMA

Exondys 51
Duchenne muscular dystrophy
(approved Sep. 2016)



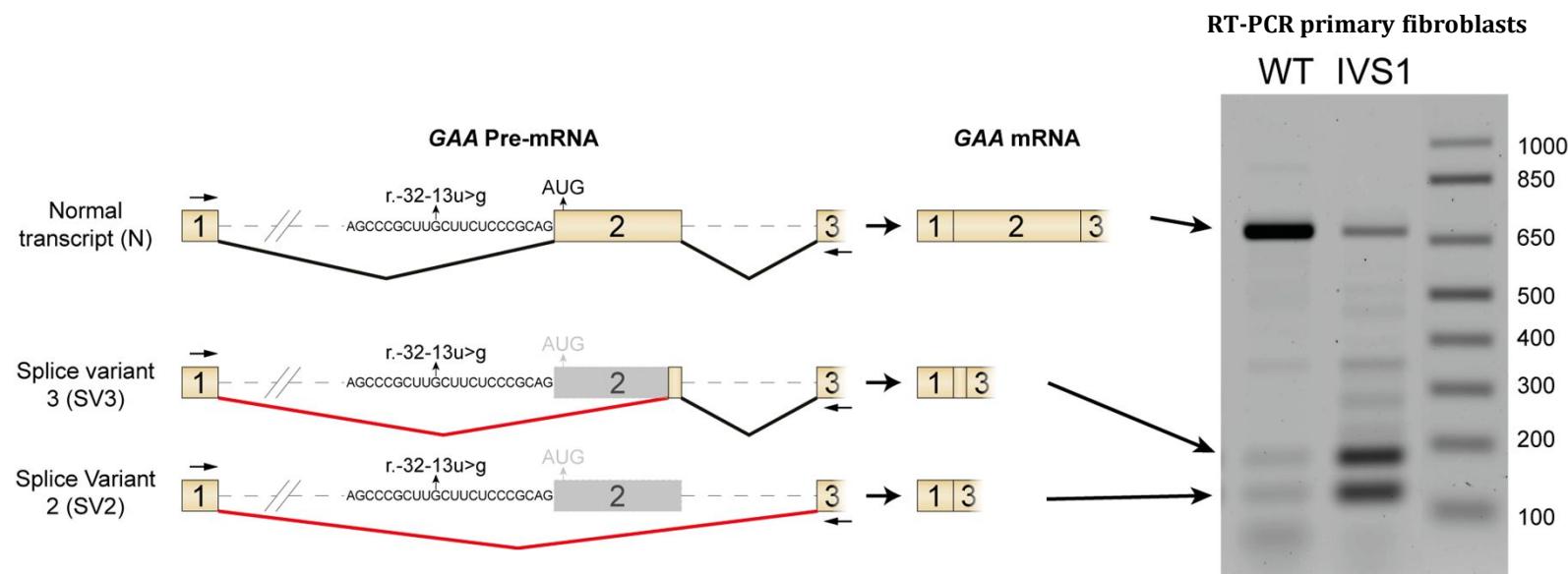
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RNA-based therapy for Pompe patients

The IVS1 mutation

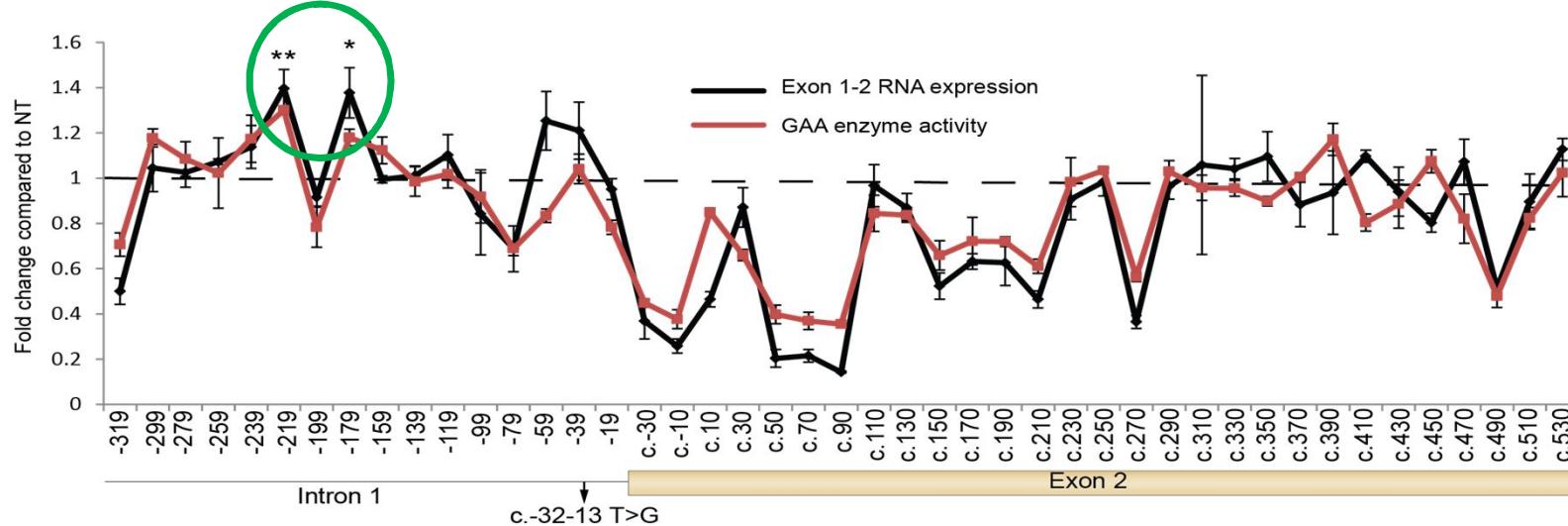
IVS1 mutation (c.-32-13T>G)

- Most common mutation in Pompe patients
- 60% of juvenile and 95% of adult Caucasian Pompe patients carry this mutation
- IVS1 is located before *GAA* exon 2 and affects splicing of that exon



Where is such a splicing silencer located?

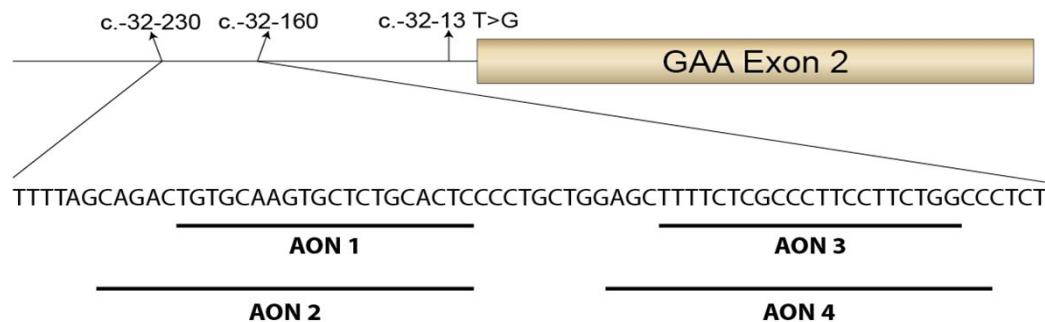
A virus-based AON screen was performed in IVS1 patient fibroblasts to find potential AON target sites.



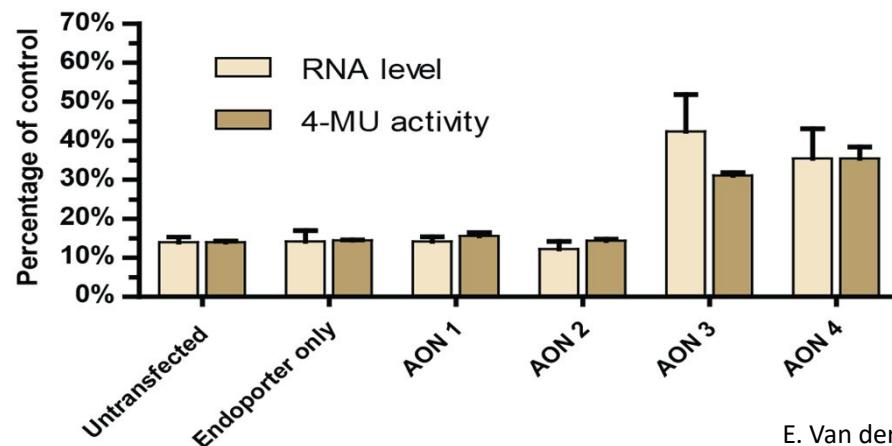
- Two potential hits were detected.
- Are these hits reproducible with therapeutical AONs?

Splicing regulatory elements

Four AONs targeting the sites detected with the screen were designed



- GAA mRNA expression and GAA enzymatic activity are above the disease threshold for AONs 3 and 4

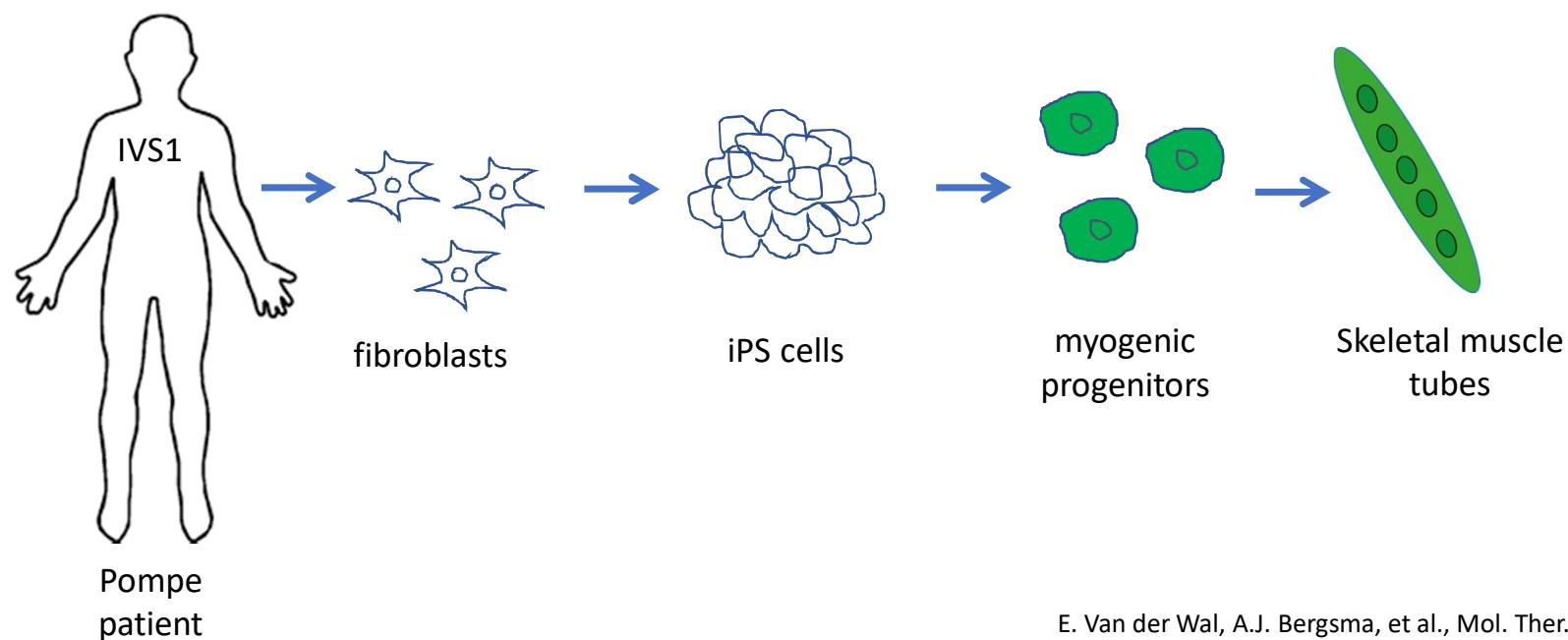


E. Van der Wal, A.J. Bergsma, et al., Mol. Ther. Nucleic acids, 2017a

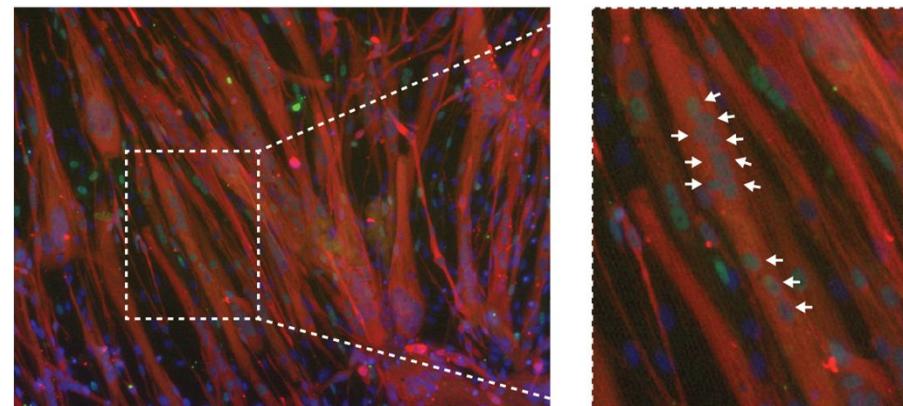
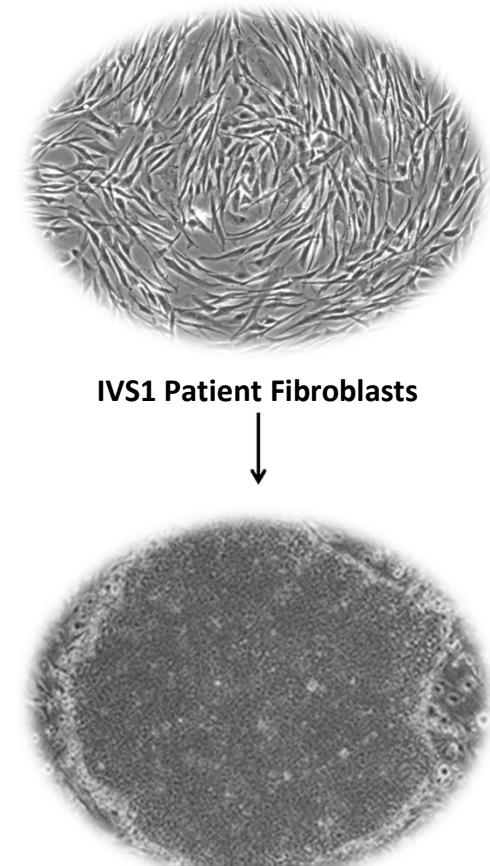
Cell-culture model for Pompe disease

Generation of skeletal muscle cells from skin cells

- The target tissue in Pompe disease is skeletal muscle cells
- Skeletal muscle cells were generated from patient skin cells (also called fibroblasts)



E. Van der Wal, A.J. Bergsma, et al., Mol. Ther. Nucleic acids, 2017b



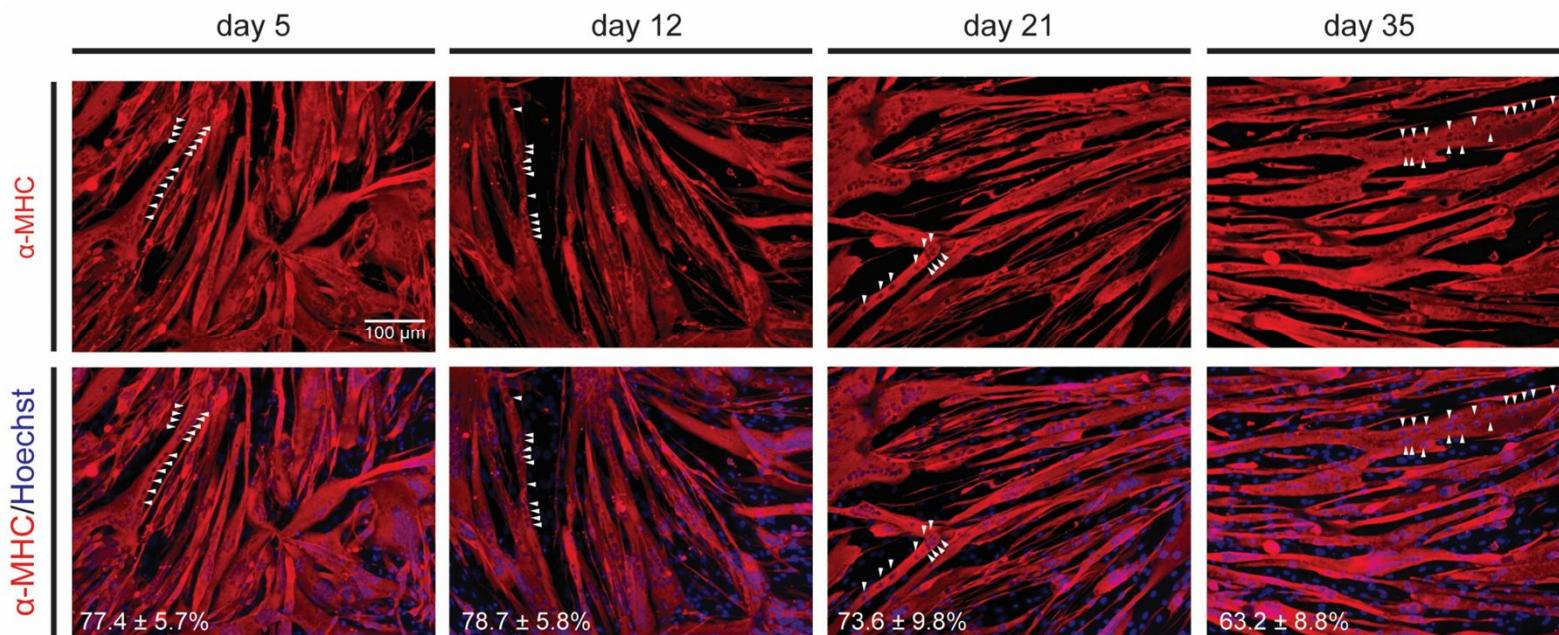
Good system for use in drug screening:

- Expansion potential: **10 million fold**
- High Reproducibility
- Freeze/thaw progenitors for easy culturing
- Fusion index >60%

Induced Pluripotent Stem cells (iPSCs)

E. Van der Wal, A.J. Bergsma, et al., Mol. Ther. Nucleic acids, 2017b

A large percentage of cells (>60%) contribute to myotube formation.



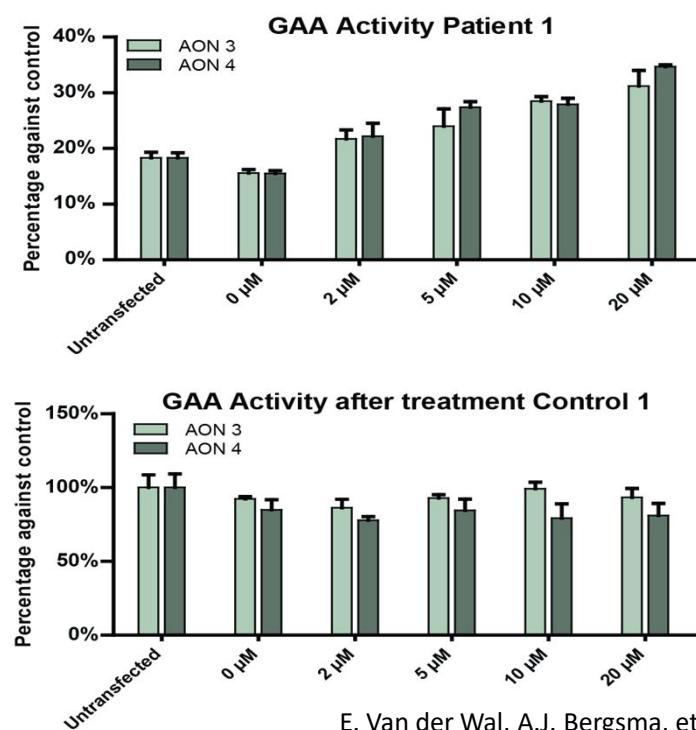
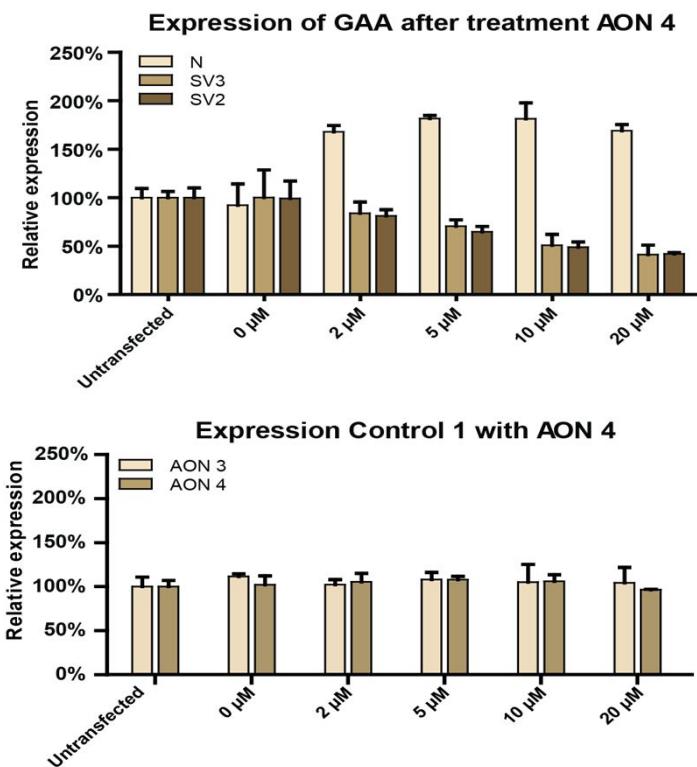
“patient in a dish”

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Testing of AON in skeletal muscle cells

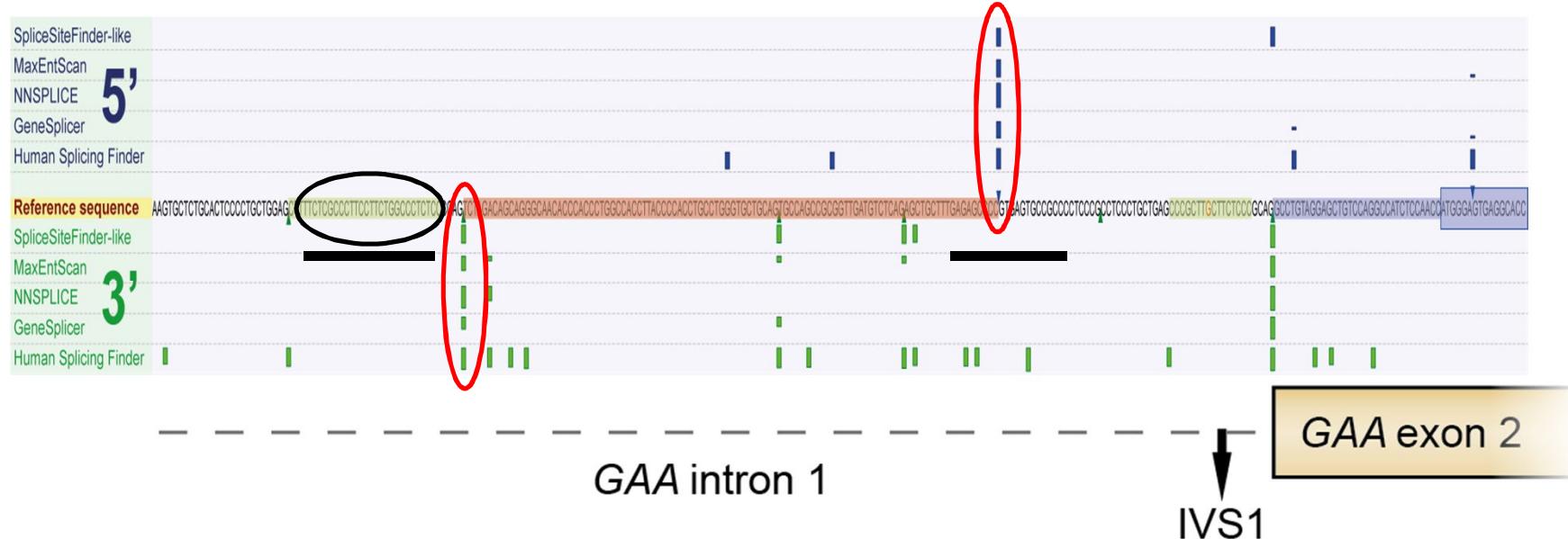
Testing of AON in skeletal muscle cells

AONs were tested in different concentration in patient derived skeletal muscle cells:



Mechanism of IVS1 pathogenicity

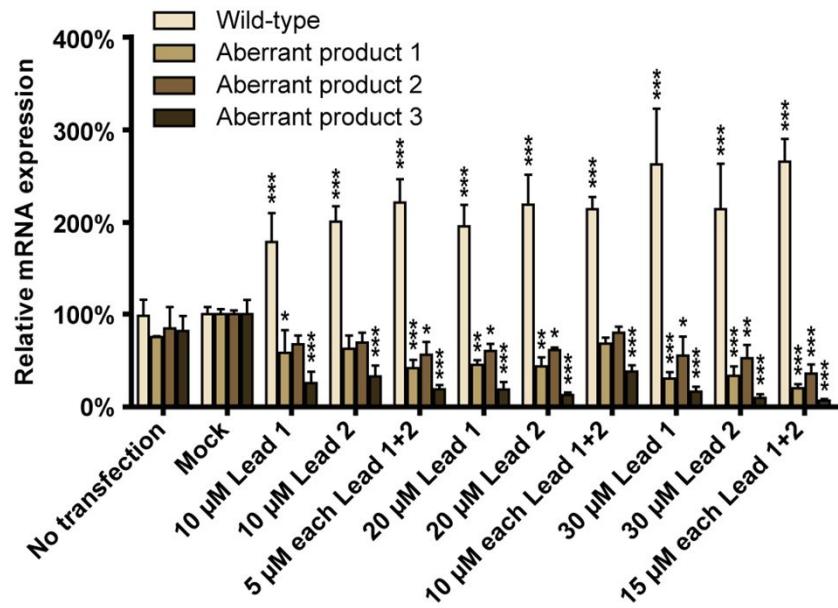
Targeted region



- Pseudo exon present in intron 1
- Block both splice sites with AONs

Generation of skeletal muscle cells from skin cells

- AONs tested in patient iPS-derived skeletal muscle cells



Combination of AONs restores:

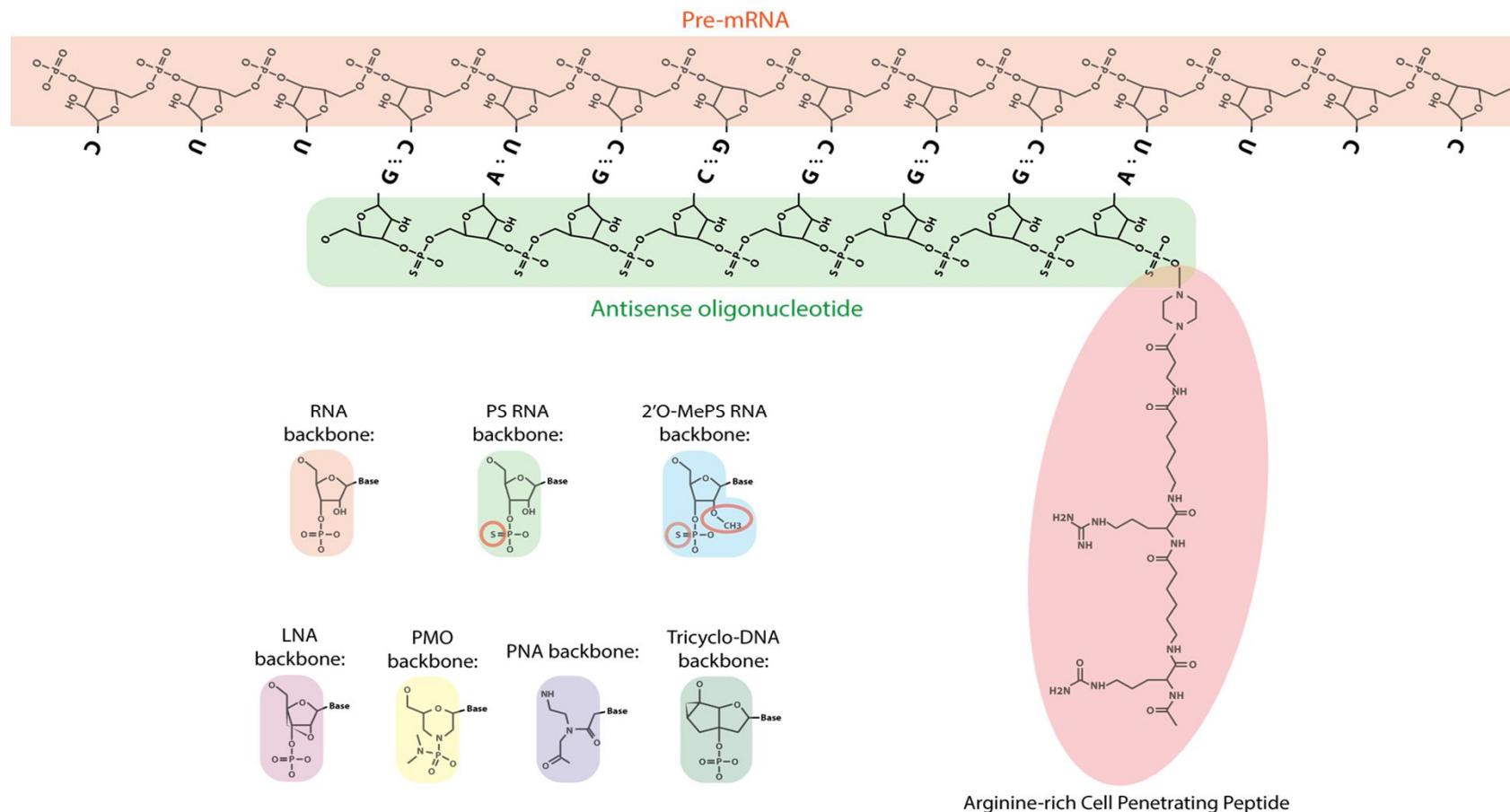
- GAA exon 2 skipping
- Almost complete rescue of GAA enzymatic activity to well above 20% (the disease threshold)

E. Van der Wal, A.J. Bergsma, et al., Mol. Ther. Nucleic acids, 2017b

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Future prospects

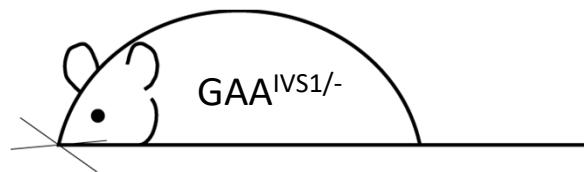
Optimization of the AONs to improve uptake and efficacy



Mouse models to model IVS1 Pompe disease

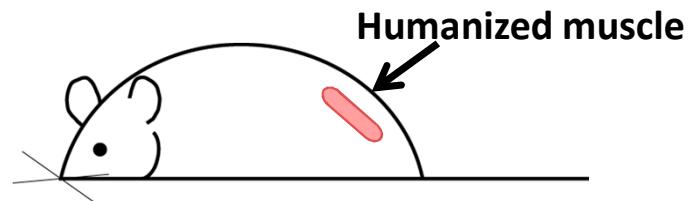
Model 1:

- Contains part of the human GAA gene
- The mouse GAA exon 1-3 is replaced with the human GAA exon 1-3 and contains the IVS1 variant



Model 2:

- This model can receive human muscle cells
- The background of this mouse is such that we can replace a mouse muscle and grow a muscle with human cells instead



Future perspectives

- Testing different types of AONs to improve uptake
- AONs have to be tested in animal models to test multiple parameters, such as:
 - Efficacy of the AONs
 - Uptake in muscle tissue
 - Pharmacodynamics and pharmacokinetics
 - Dose response
 - Safety
- If above parameters are successfully optimized, clinical trials can be started

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Conclusions

Summary

- Although ERT is available for Pompe patients, there is a need for new treatment strategies.
- RNA-based therapy could benefit more than 75% of Pompe patients.
- We developed an AON mediated therapy that almost fully restores aberrant splicing caused by the IVS1 variant.
- New AONs are being developed to rescue aberrant splicing caused by other mutations in the GAA gene.
- Further *in vitro* and *in vivo* testing and optimisation is currently being performed.
- Positive findings in these experiments should lead to the start of clinical trials.

Acknowledgements

Special thanks to:

Dr. W.W.M. Pim Pijnappel

Prof. Dr. Ans T. van der Ploeg

Erik van der Wal

Stijn in 't Groen

Tom van Gestel

Joon Pijnenburg



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We have done a lot, a lot more can be done

The team of the Center for Lysosomal and Metabolic Diseases

