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Molecular Characterization of Portuguese Patients with pathologies related to the Lysosomal Multienzymatic Complex: Sialidosis and Galactosialidosis

INSTITUTO NACIONAL DE SAÚDE
Dr. Ricardo Jorge

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2009

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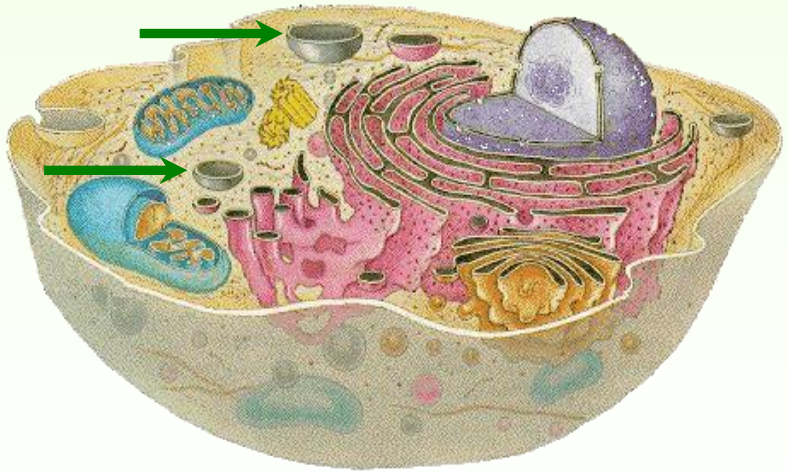
I. Introduction

1. Lysosomes

- Cytoplasmic organelles
- 50 – 60 hydrolytic enzymes

- Major function –

intracellular digestion



Eukaryothic cell structure with several lysosomes indicated.
http://www.prof2000.pt/users/pjgameiro/ESMCBiologia/celula.gif

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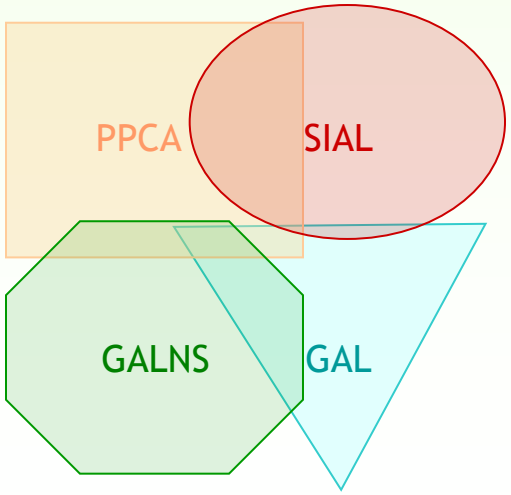
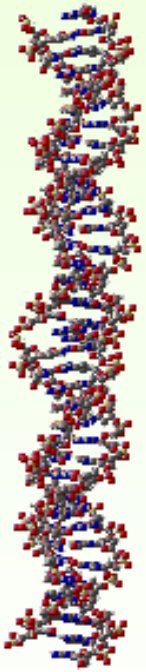
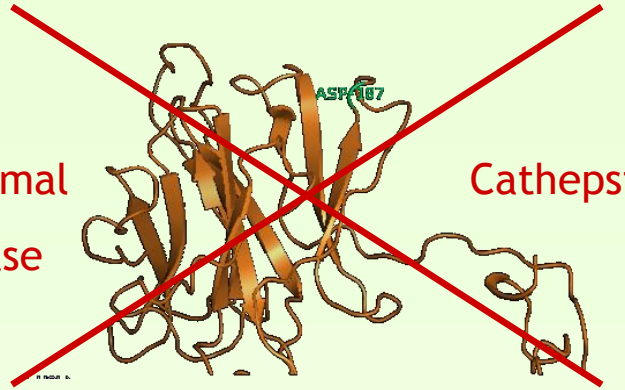
2. The Lysosomal Multienzyme Complex (LMC)

- Functional association of four enzymes:
  - Cathepsin A (PPCA)
  - Sialidase (SIAL)
  - β-galactosidase (GAL)
  - N-acetylaminogalacto-6-sulfate sulfatase (GALNS)





Lysosomal sialidase



Cathepsin A

Intralysosomal storage of these enzyme's substrates

Sialidosis Pathological condition **Galactosialidosis**

- Clinical phenotypes:
  - Early infantile
  - Late infantile
  - Juvenile/adult

Severity

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II. Objectives

- identify and characterize the mutations present in the Portuguese patients with each one of these pathologies;

- develop methods for detection of S and GS alleles
  - molecular diagnosis strategies
  - pre-natal diagnosis
  - carrier detection

- study the effect of each mutations at mRNA level;

- evaluate the impact of each mutation on the structure/function of the respective protein.

- establish a genotype-phenotype correlation.

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III. Methods

Studied patients

7 Portuguese patients,

Galactosialidosis – 4;

Sialidosis – 3.

Diagnosis previously confirmed through biochemical methods

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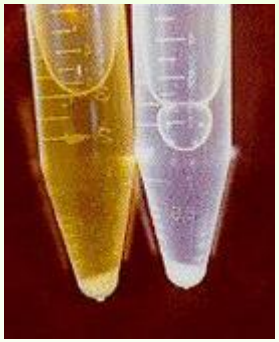
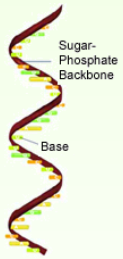
Fibroblasts cell culture and pelletization



RNA extraction



DNA extraction

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Mutational analysis of *NEU1* and *PPGB* genes

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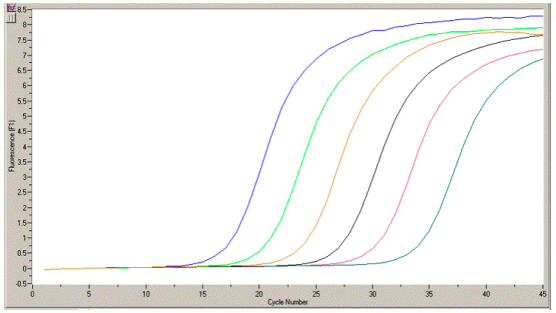
Expression studies of *NEU1* and *PPGB* genes
(mRNA quantification)

qtRT-PCR

(quantitative real time PCR)



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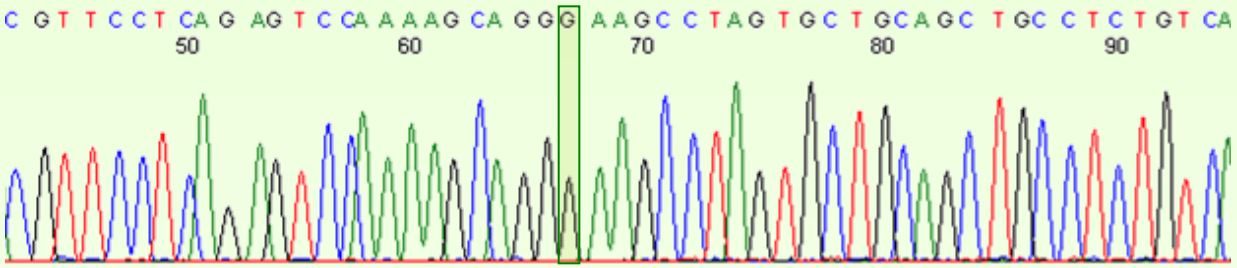
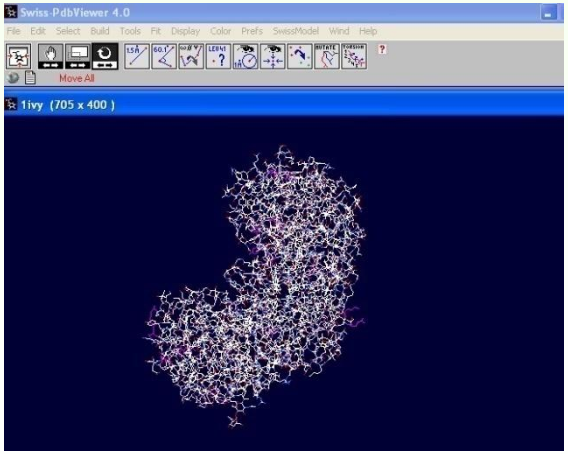
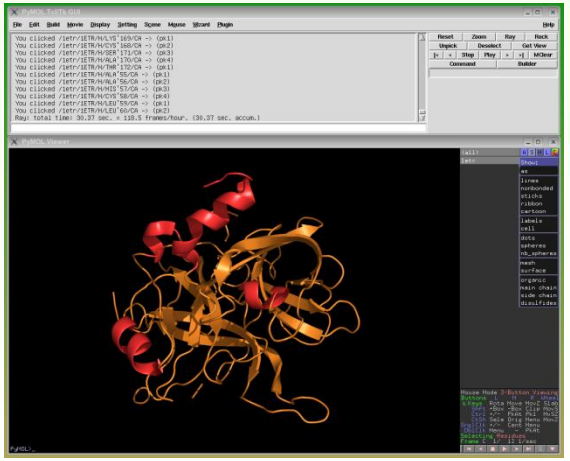
Three-dimensional analysis of the novel missense mutations found in the *NEU1* and *PPGB* genes







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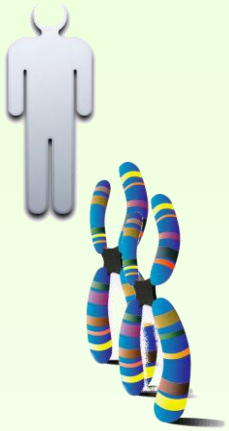
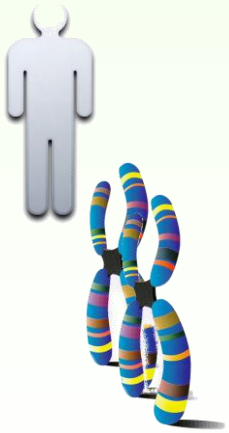
IV. Results and Discussion

1. Mutations found in the *NEU1* and *PPGB* genes



- **Sialidosis Patients**
  - 3 novel missense mutations
    (D187N; P153L; Q235H)
  - 1 novel nonsense mutation
    (R341X)



- **Galactosialidosis Patients**
  - 2 novel deletions
    (c.228-229delC; c.991-992delT)
  - 1 novel missense mutation
    (G57V)
  - 1 reported missense mutation
    (V104M)

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2.1. Mutations found in the *NEU1* gene

3 missense mutations + 1 nonsense mutation

- All these mutations were analysed trough bioinformathic tools

- In order to get a first insight on the significance of missense alterations in protein function
  - PolyPhen
  - SIFT.

- Multiple sequence alignments were performed in order to verify the degree of conservation of the affected residues

```
VCGHGTLERDGVFCLLSDDHGV
VCGHGTLERDGVFCLLSDDHGA
VCGHGTLERDGVFCLLSDDHGA
VCGHGTLERDGVFCLLSDDHGA
VCGHGTLERDGVFCLLSDDHGA
VCGHGTLERDGVFCLLSDDHGA
VCGHGTLERDGVFCLLSDDHGA
VCGHRTLEQDGVFCLLSDDHGA
VCGHGTLERDGVFCLLSDDHGA
VCGHGTLERDGVFCLLSDDHGA
VCGHGTLERDGVFCLLSDDHGD
VCGHGTLERDGVFCLLSDDHGA
VCGHGTLKRDGVSCLISDDKGA
VCGHGTIAGDGVFCILSDDHGR
```

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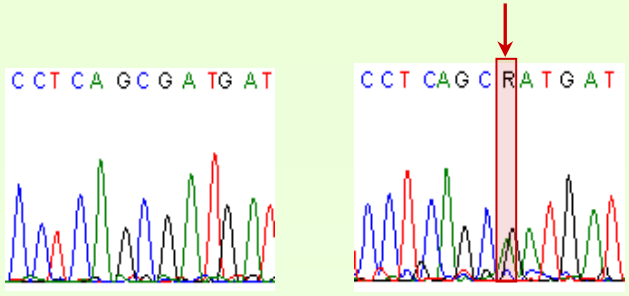
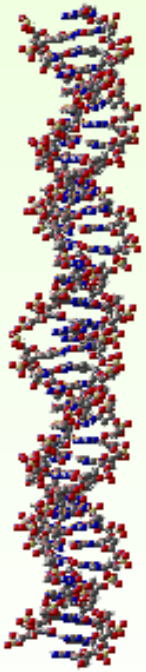
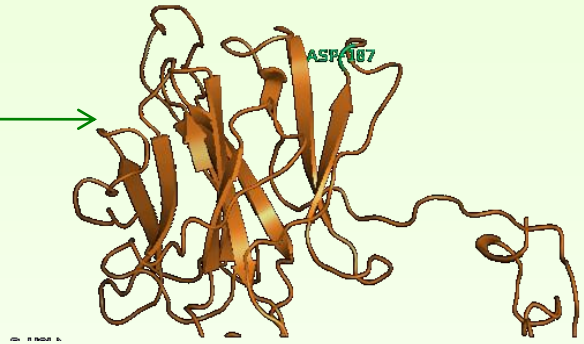


D187N

Hard to understand!

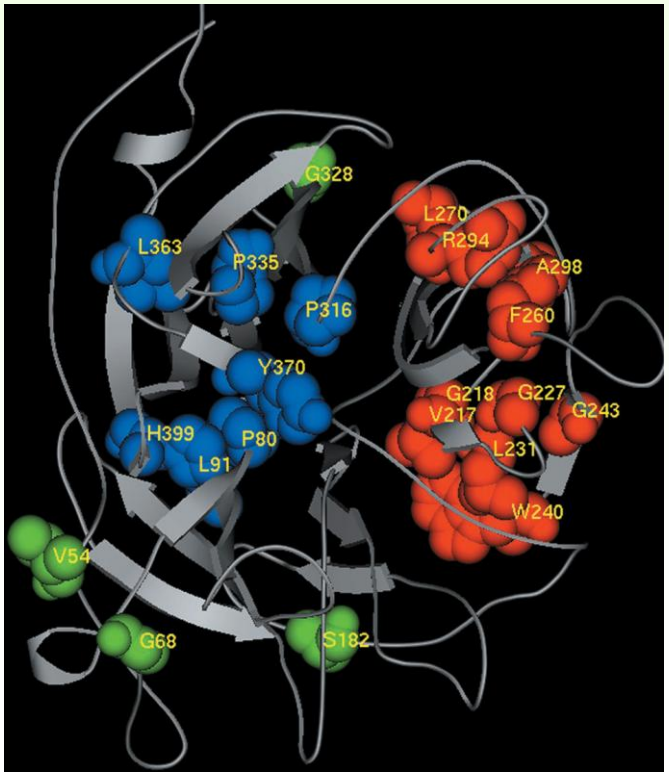
Absence of 3D modeling in the surrounding regions





However…

This mutation seems similar to a group of genetic alterations already mentioned by Lukong *et al*. (2001), when they analyzed the impact of several sialidosis-causing mutations: the ones that do not cause obvious structural changes.



(figure: represented *in green*)

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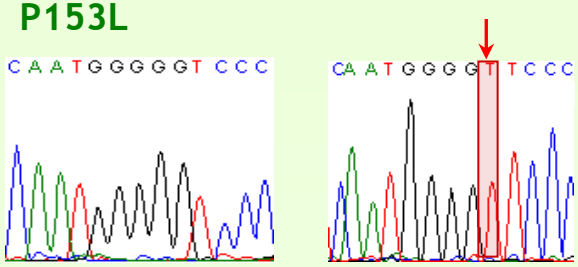
P153L



Mutations located in this region are associated with a dramatic reduction of the enzyme activity

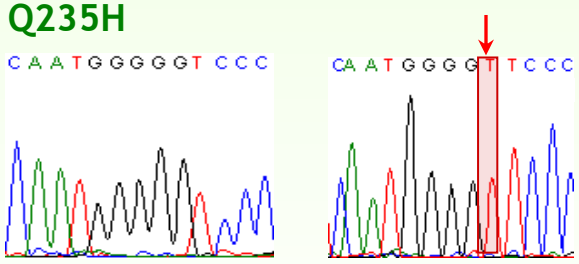
pathological condition

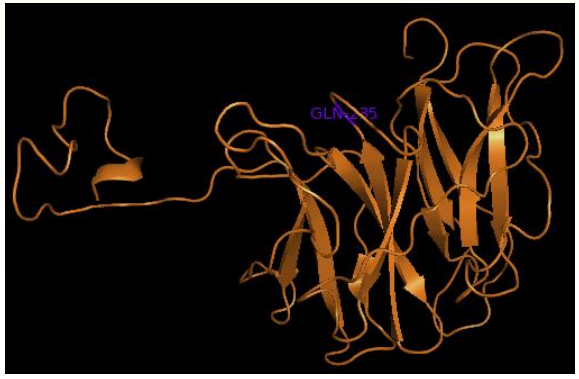
Both residues are located in one region on the surface of the molecule

sialidase binding interface with cathepsin A

- crucial -

Q235H





Missense

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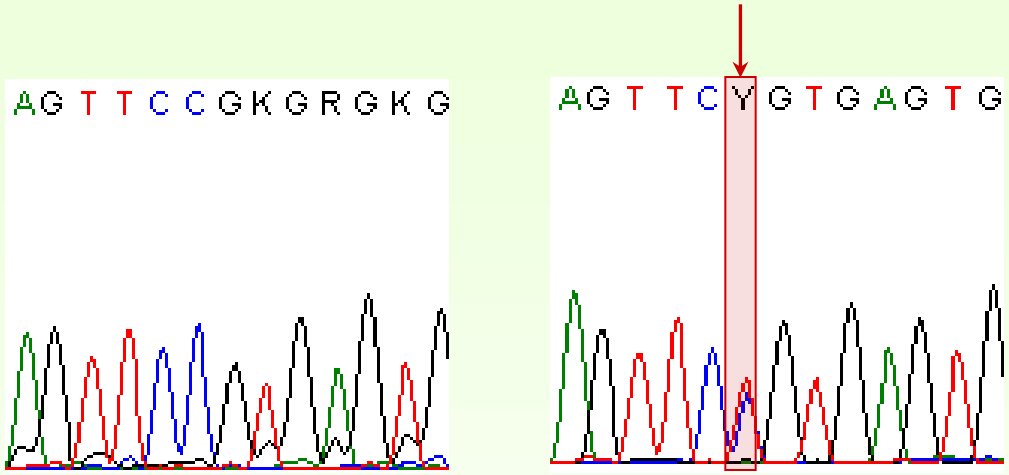
Nonsense mutation (R341X)



- Consequence: formation of a truncated protein

. introduction of a premature STOP codon downstream

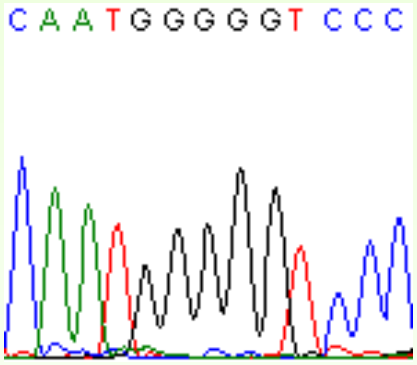
. - 74 aa less than the normal protein

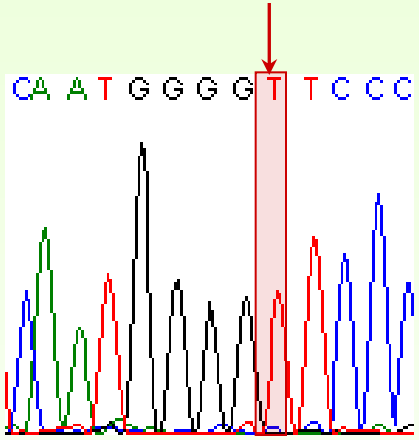
Nonsense

2.1. Mutations found in the *PPGB* gene

2 deletions + 1 missense mutation

G57V





- c.254 G → T
- Conserved region
- Bioinformatic predictions – deleterious
  - SIFT - 0,00 (non tolerated)
  - PolyPhen – probably deleterious

```
ENSPVVLWLNGGPGCSSLDGLI
ENSPVVLWLNGGPGCSSLDGLI
ENSPVVLWLNGGPGCSSLDGLI
ENSPVVLWLNGGPGCSSLDGLI
KSSPVVLWLNGGPGCSSLDGLI
KSSPVVLWLNGGPGCSSLDGLI
KSSPLVLWLNGGPGCSSLDGFI
KSSPVELWLNGGPGCSSLDGFI
KTSPLVLWLNGGPGCSSLDGLI
KNSPVVLWLNGGPGCSSLDGLI
KSSPVVLWLNGGPGCSSLDGX
QSSPVVLWLNGGPGCSSLDGLI
NNSPVVLWLNGGPGCSSLDGLI
VSSPVVLWLNGGPGCSSMDGLI
STDPVLLWLTGGPGCSGLSALI
```

Missense

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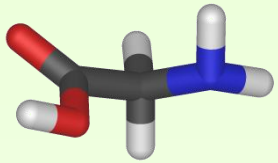
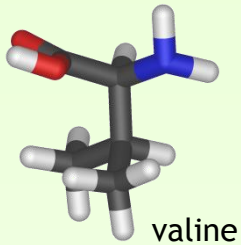
Three-dimensional analysis of the effect of mutation G57V



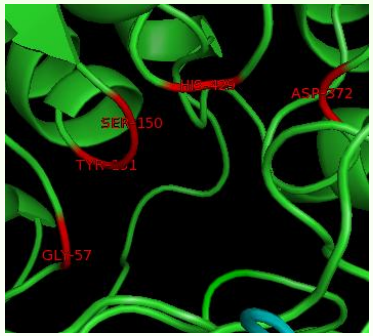
glicine



valine

- very unique amino acid
- > conformational flexibility

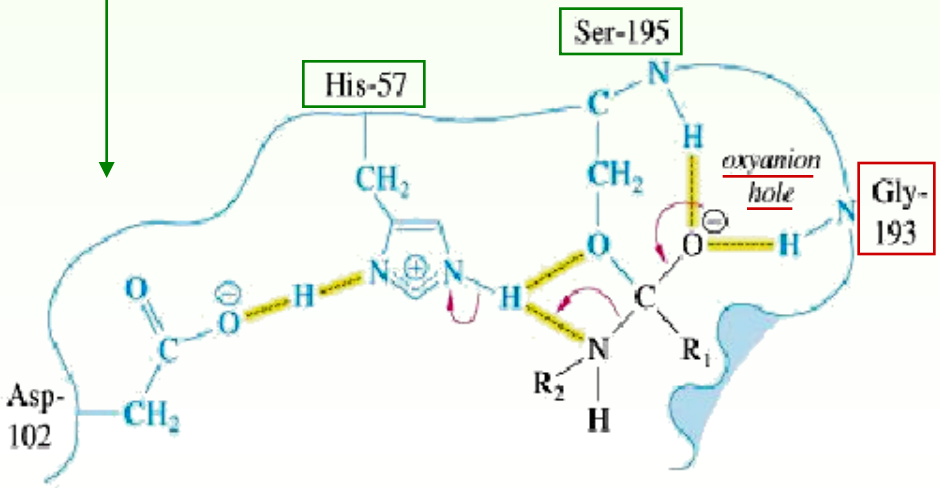
But…



Cathepsin A

active site

E-TI$_1$



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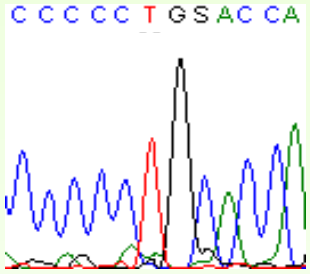
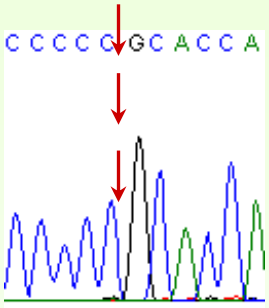
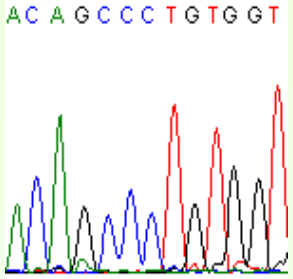
Deletions (c.228-229delC; c.991-992delT)

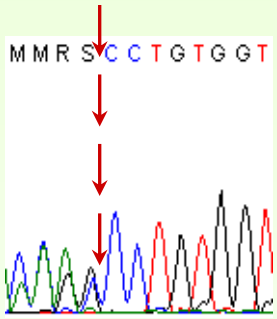
c.228-229delC





c.991-992delT





- Consequence: frameshift mutation

↓

formation of a truncated protein

↓

. premature termination codon

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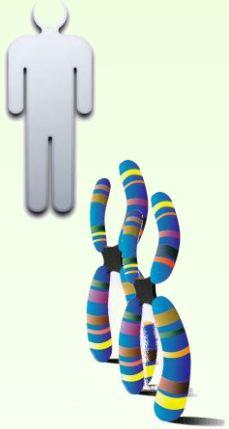
3. Impact of deletions on mRNA *PPGB* levels

3.1. General Conclusions



Patient GS3: homozygous c.991-992delT

Reduced levels of mRNA *PPBG*

Why?!

c.991-992delT

↓

***P**remature **T**ermination **C**odon*

↓

Nonsense mediated mRNA decay

4. Genotype-phenotype correlation

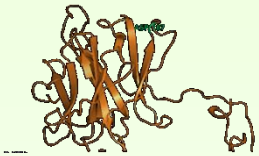
4.1. Sialidosis Patients

S1

D187N/

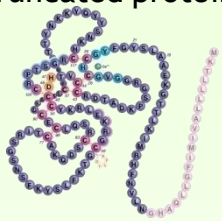
R341X

Doesn't cause obvious



structural changes

Truncated protein



NMD

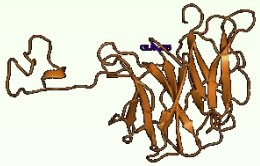
Sialidosis type I

S3

Q235H/

Q235H

Bioinformatic prediction: tolerated



NEU1/cathepsin binding site

S2

P153L/

P153L

NEU1/PPCA

sinding site

Sialidosis type II

4. Genotype-phenotype correlation

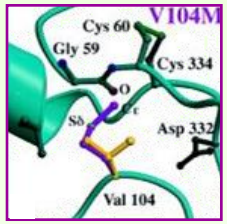
4.1. Galactosialidosis Patients

GS1

V104M/

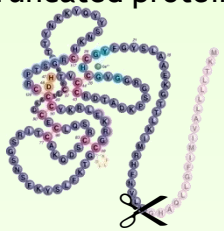
228-229delC

Severe form of the disease



(Zhou *et al.*, 1996)

Truncated protein



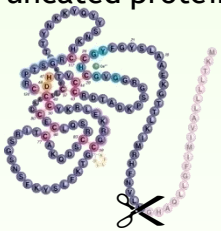
NMD

GS3

991-992delT/

991-992delT

Truncated protein



NMD

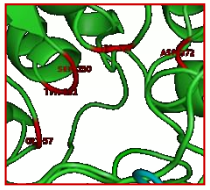
Early infantile type

GS4

G57V/

G57V

G57V



Cathepsin A active site

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V. Conclusions

In conclusion, it was possible to:

- identify and characterize the mutations present in the Portuguese patients with both pathologies;
- contribute to a better diagnosis of sialidosis and galactosialidosis;
- evaluate the impact of each mutation not only in the function but also in the three dimensional structure of each protein;
- get an insight on the impact of deletions on mRNA levels;
- establish a genotype-phenotype correlation.

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Thank you very much!

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