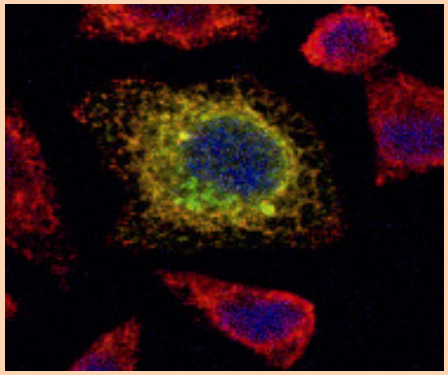
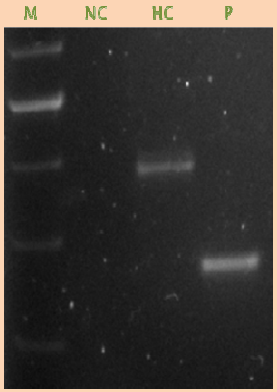
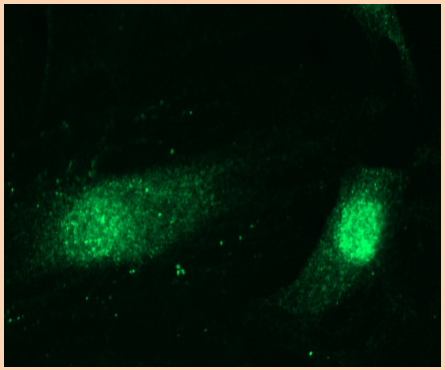
Jornadas CECA 2014







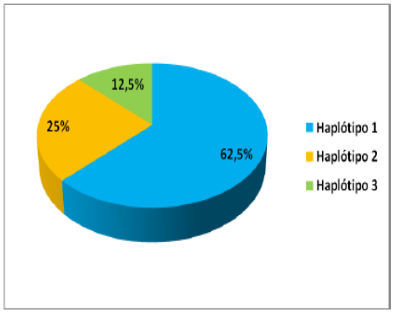
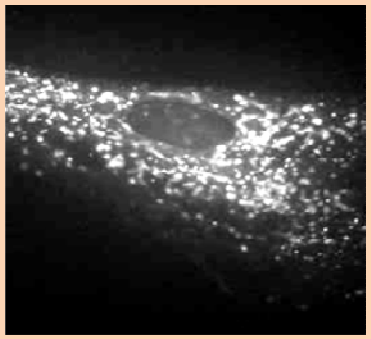
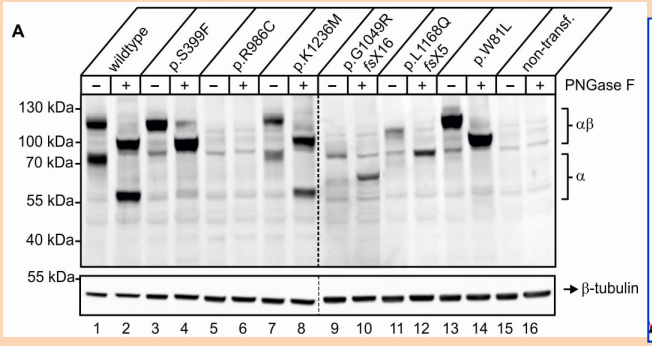


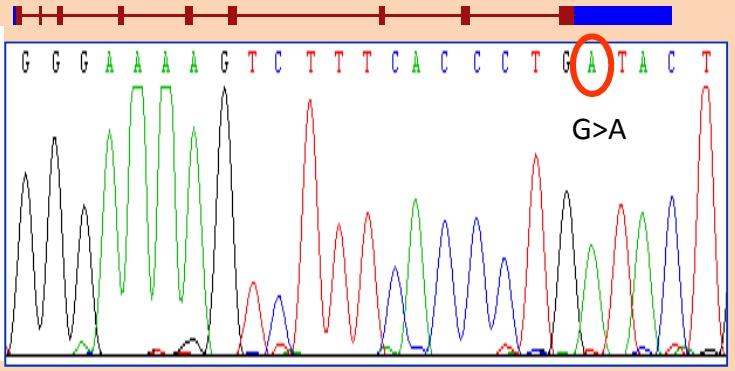
Figura 9 – Gráfico com as percentagens obtidas dos diferentes haplótipos encontrados nos portadores da mutação W402X.

Lysosomal Storage Diseases: pathophysiology and innovative therapeutic approaches

Sandra Alves - INSA







Instituto_Nacional de Saúde | 1899 | www.insa.pt

UID - Departamento de Genética Humana

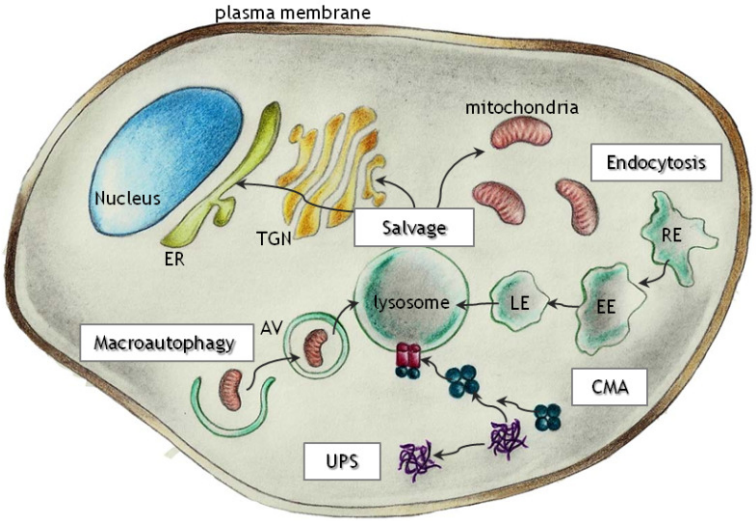
Group members

Sandra Alves, Olga Amaral, Francisca Coutinho, Liliana Matos, Ana Joana Duarte, Diogo Ribeiro.

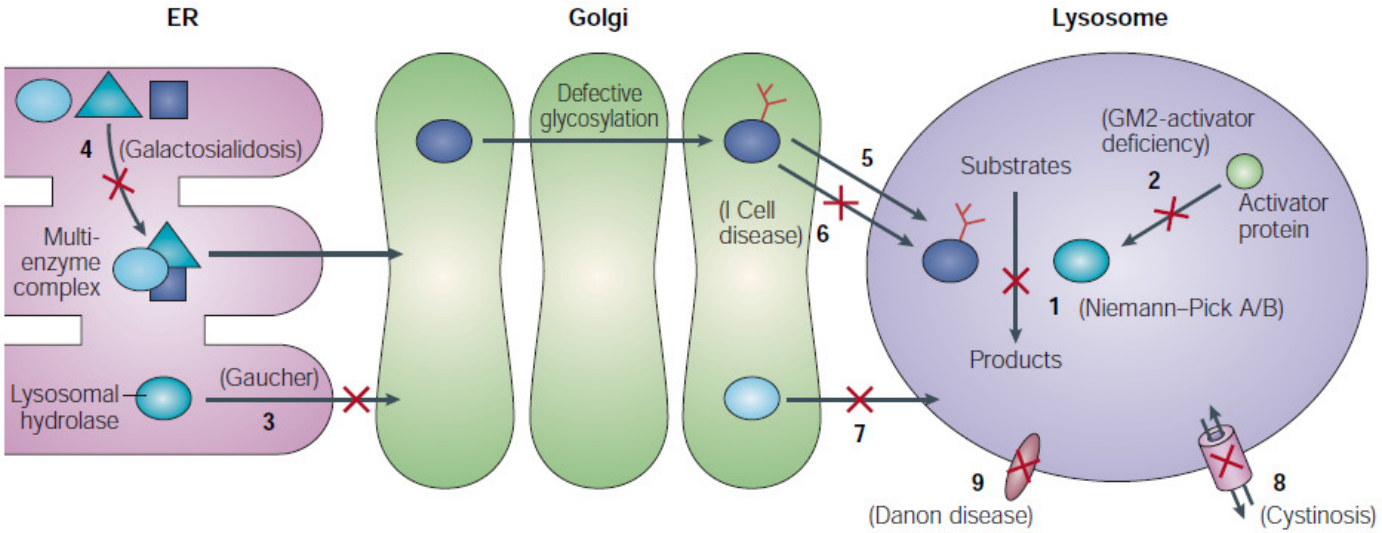
Colaborations:

- Maria João Prata - IPATIMUP.
- Belén Pérez. Centro de Biología Molecular Severo Ochoa Nicolas Cabrera, Universidad Autónoma de Madrid.
- Daniel Grinberg. Centro de investigação Biomédica em Rede de Doenças Raras (CIBERER). Barcelona.
- Thomas Braulke, Department of Biochemistry, University Medical Center Hamburg-Eppendorf.
- Alexey Pshezhetsky - CHU Sainte-Justine, Montreal, Canada

Lysosome – central role in cell metabolism



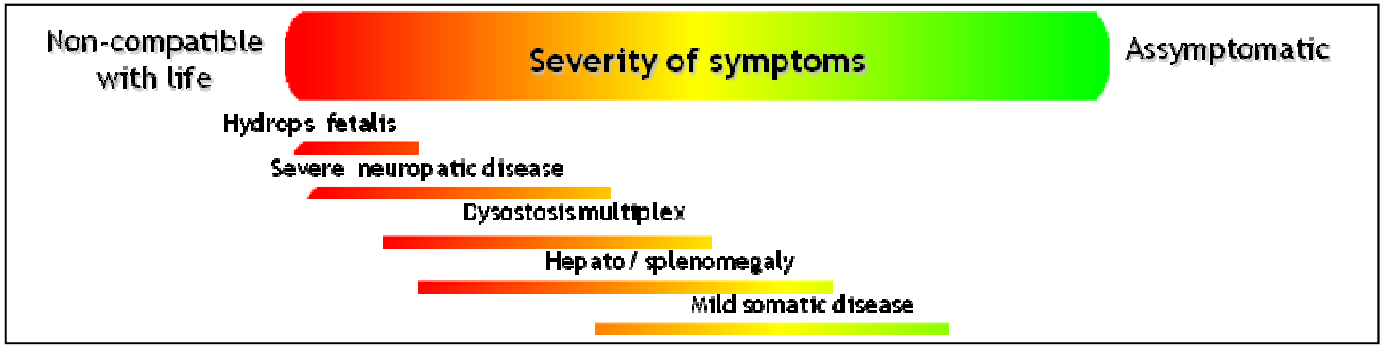
The biochemical and cellular basis of lysosomal storage disorders



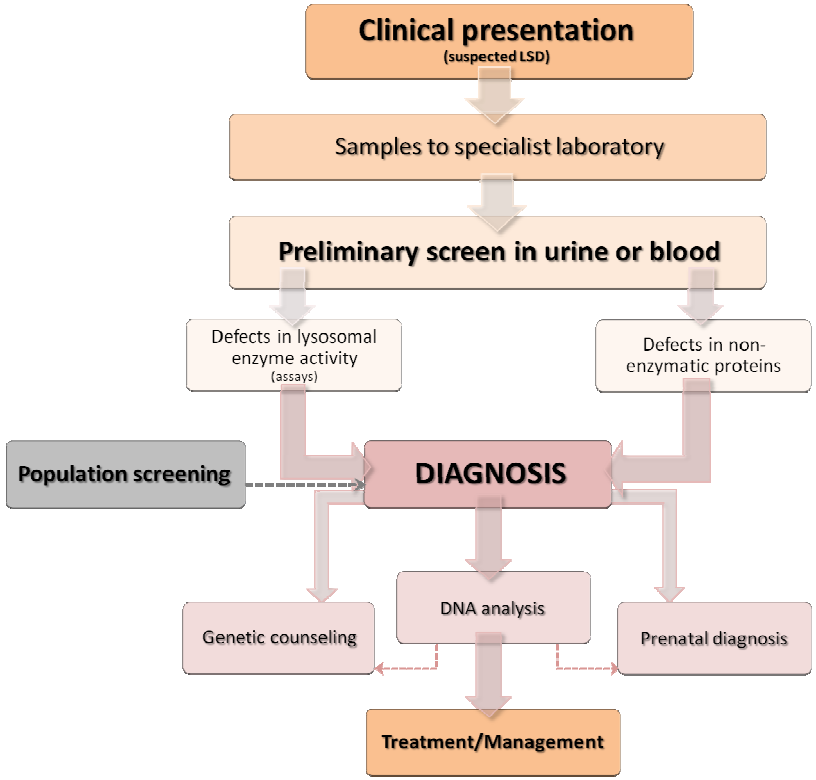
Disease	Defective protein	Main storage materials
Sphingolipidoses		
Fabry	α-Galactosidase A	Globotriasylceramide and blood-group-B substances
Farber lipogranulomatosis	Ceramidase	Ceramide
Gaucher	β-Glucosidase Saposin-C activator	Glucosylceramide Glucosylceramide
Niemann–Pick A and B	Sphingomyelinase	Sphingomyelin
Sphingolipid-activator deficiency	Sphingolipid activator	Glycolipids
GM1 gangliosidosis	β-Galactosidase	GM1 ganglioside
GM2 gangliosidosis (Tay–Sachs)	β-Hexosaminidase A	GM2 ganglioside and related glycolipids
GM2 gangliosidosis (Sandhoff)	β-Hexosaminidase A and B	GM2 ganglioside and related glycolipids
GM2 gangliosidosis (GM2-activator deficiency)	GM2-activator protein	GM2 ganglioside and related glycolipids
Mucopolysaccharidoses (MPS)		
MPS I (Hurler, Scheie, Hurler/Scheie)	α-Iduronidase	Dermatan sulphate and heparan sulphate
MPS II (Hunter)	Iduronate-2-sulphatase	Dermatan sulphate and heparan sulphate
MPS IIIA (Sanfilippo)	Heparan *N*-sulphatase (sulphamidase)	Heparan sulphate
MPS IIIB (Sanfilippo)	*N*-Acetyl-α-glucosaminidase	Heparan sulphate
MPS IIIC (Sanfilippo)	Acetyl-CoA:α-glucosamide *N*-acetyltransferase	Heparan sulphate
MPS IIID (Sanfilippo)	*N*-Acetylglucosamine-6-sulphatase	Heparan sulphate
Morquio-A disease	*N*-Acetylgalactosamine -6-sulphate-sulphatase	Keratan sulphate, chondroitin-6-sulphate
Morquio-B disease	β-Galactosidase	Keratan sulphate
MPS VI (Maroteaux–Lamy)	*N*-Acetylgalactosamine-4-sulphatase (arylsulphatase B)	Dermatan sulphate
MPS VII (Sly)	β-Glucuronidase	Heparan sulphate, dermatan sulphate, chondroitin-4- and -6-sulphates
Oligosaccharidoses and glycoproteinosis		
Pompe (glycogen-storage-disease type II)	α-Glucosidase	Glycogen

Disease	Defective protein	Main storage materials
Diseases caused by defects in integral membrane proteins		
Cystinosis	Cystinosin	Cystine
Danon disease	LAMP2	Cytoplasmic debris and glycogen
Infantile sialic-acid-storage disease and Salla disease	Sialin	Sialic acid
Mucoplipidosis (ML) IV	Mucolipin-1	Lipids and acid mucopolysaccharides
Niemann–Pick C (NPC)	NPC1 and 2[‡]	Cholesterol and sphingolipids
Others		
Galactosialidosis	Cathepsin A	Sialyloligosaccharides
I Cell and pseudo-Hurler polydystrophy (ML II and ML III, respectively)[§]	UDP-*N*-acetylglucosamine:lysosomal enzyme *N*-acetylglucosaminyl-1-phosphotransferase	Oligosaccharides, mucopolysaccharides and lipids
Multiple sulphatase deficiency	Cα-formylglycine-generating enzyme	Sulphatides
Neuronal ceroid lipofuscinosis (NCL)1 (Batten disease)[‖]	CLN1 (protein palmitoylthioesterase-1)	Lipidated thioesters
NCL2 (Batten disease)	CLN2 (tripeptidyl amino peptidase-1)	Subunit c of the mitochondrial ATP synthase
NCL3 (Batten disease)	Arginine transporter	Subunit c of the mitochondrial ATP synthase

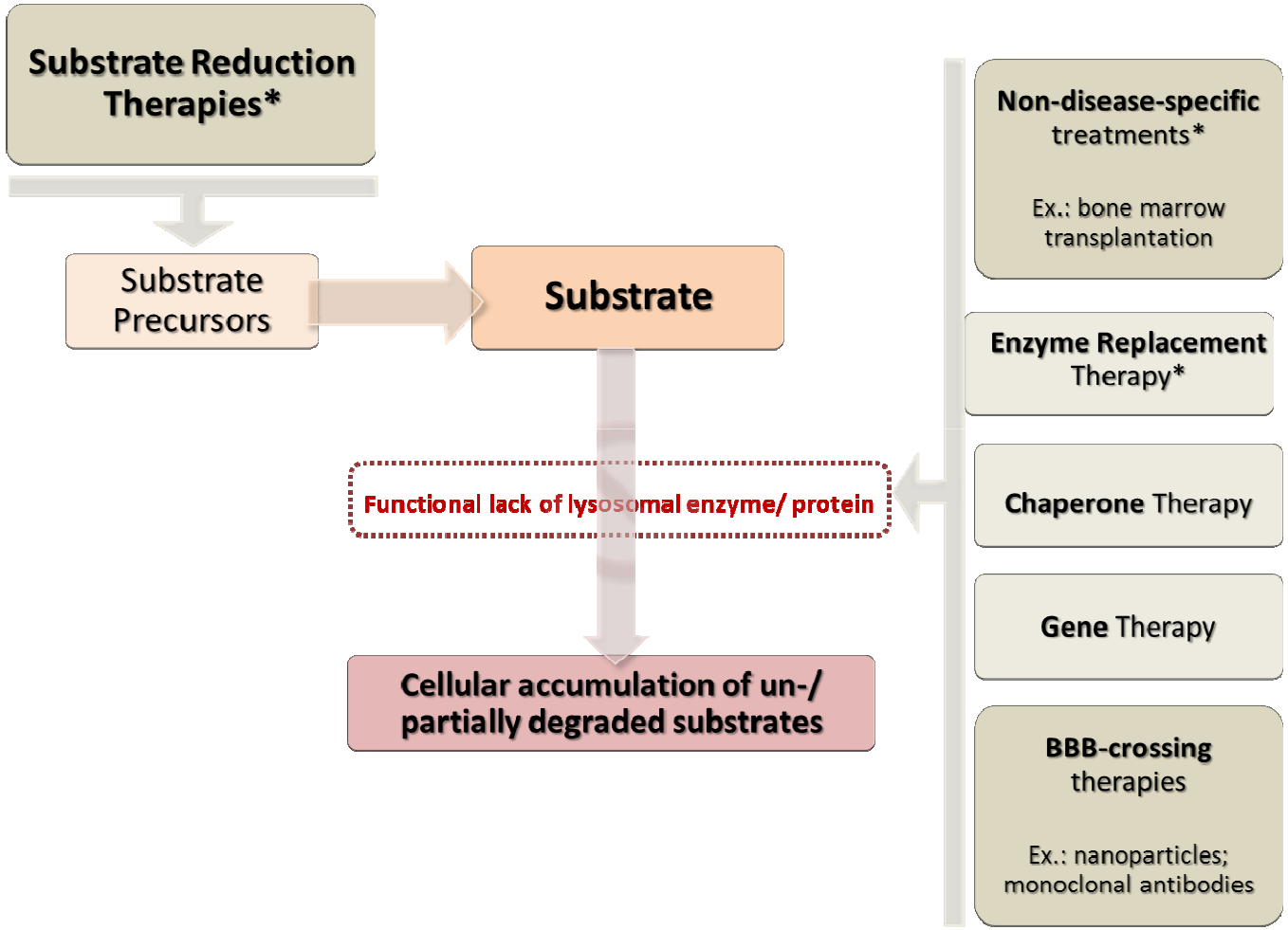
Clinical Symptoms



Diagnosis



Lysosomal Disorders and Common Therapeutic Approaches



Research Lines/Projects

Molecular and cellular characterization of lysosomal diseases: diversity, frequency and causality

•"Prognosis and prevention in a few inherited diseases existing in the Portuguese population" PIC/IC/82822/2007.

• "Molecular analysis of the mucolipidosis II and III in Portugal: characterization of the mutational spectrum and relationship with clinical phenotypes". PIC/IC/83252/2007

•"The sorting and trafficking of lysosomal proteins through M6P independent pathways: molecular, biochemical and functional studies". PTDC/SAL-GMG/102889/2008

Development of alternative therapeutic approaches

• "Splicing therapeutics for patients affected by a lysossomal storage disorders". (FCT SFRH/BD/64592/2009). "

• Development of a U1 snRNA-adapted gene therapeutic strategy to correct 5' splicing defects in lysosomal storage disorders". SPDM/Genzyme Grant.

• Less is more - Substrate Reduction Therapy for Mucopolysaccharidoses through RNAi. Fundação Millennium bcp grant.

• Cost action BM1207 – Networking towards application of antisense mediated exon skipping (2013-2017).

Other projects

• Use of next generation sequencing, for the study of patients presenting symptoms frequently associated with LSDs, but without a specific diagnosis.

• Development of iPSCs as disease specific cellular models for LSDs

Mucopolysaccharidoses type II

16 unrelated Portuguese patients;

15 different mutations;

9 of the 15 affected the usual splicing pattern at the locus.

J Inherit Metab Dis (2006) 29:743–754
DOI 10.1007/s10545-006-0403-z

ORIGINAL ARTICLE

Molecular characterization of Portuguese patients with mucopolysaccharidosis type II shows evidence that the *IDS* gene is prone to splicing mutations

S. Alves · M. Mangas · M. J. Prata · G. Ribeiro · L. Lopes · H. Ribeiro · J. Pinto-Basto · M. Reis Lima · L. Lacerda

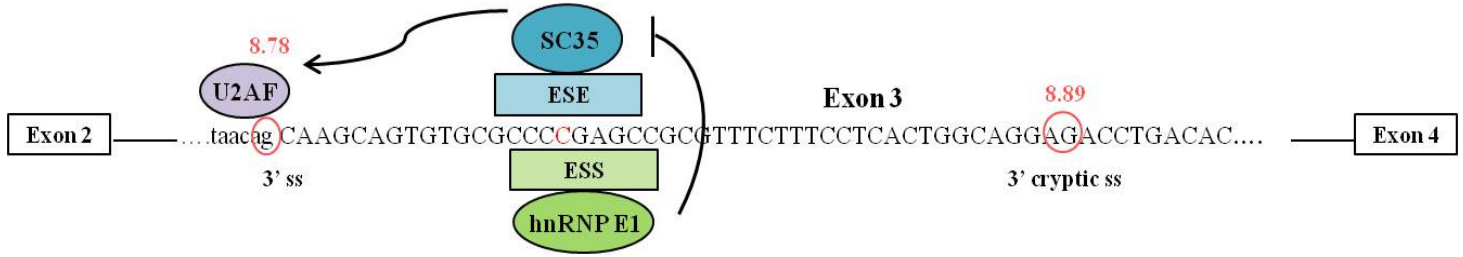
Functional analysis of IDS splicing defects

IDS gene presents alternative splicing: 11 different transcripts

Complex underlying regulatory mechanism that probably requires the interaction of multiple splicing factors, and make this gene prone to the occurrence of mutations that disrupt splicing

c.257C>T and c.241C>T (exon 3, *IDS* gene)

Through overexpression or depletion assays, we experimentally demonstrated that **SC35** and **hnRNP E1** proteins are involved respectively, in the use and repression of the constitutive 3' splice-site of exon 3.



Matos L et al. (2014) under preparation

Molecular and cellular characterization of lysosomal diseases: diversity, frequency and causality

Mucopolysaccharidoses type IIIA e IIIB

11 Portuguese patients;

5 novel mutations.

Frequent mutation: R234C (also in Spanish patients)

Haplotypic analysis: sharing of the an ancestral haplotype by Portuguese and Spanish patients - a common origin of the mutation in Iberia (recent).

Clin Genet 2008: 73: 251–256
Printed in Singapore. All rights reserved

CLINICAL GENETICS
doi: 10.1111/j.1399-0004.2007.00951.x

Short Report

Molecular analysis of mucopolysaccharidosis type IIIB in Portugal: evidence of a single origin for a common mutation (R234C) in the Iberian Peninsula

Mucopolysaccharidoses type IIIC

3 patients;

2 novel mutations.

Clin Genet 2008: 74: 194–195
Printed in Singapore. All rights reserved

CLINICAL GENETICS
doi: 10.1111/j.1399-0004.2008.01040.x

Letter to the Editor

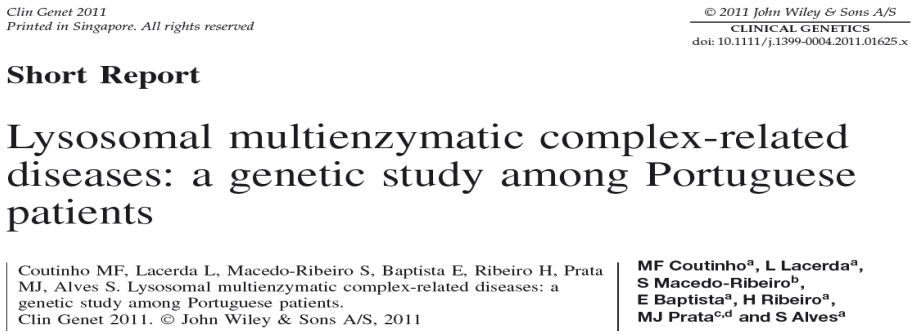
Molecular characterization of Portuguese patients with mucopolysaccharidosis IIIC: two novel mutations in the *HGSNAT* gene

Sialidosis Galactosialidosis and Glangliosidosis type 1

21 Portuguese patients studied:

- 3 affected with sialidosis (*NEU1*) ,
- 4 with Galactosialidosis (*PPGB*) and
- 14 with Glangliosidosis type 1 (*GLB1*)

Several novel missense mutations were reported



Clin Genet 2011
Printed in Singapore. All rights reserved

CLINICAL GENETICS
doi: 10.1111/j.1399-0004.2011.01625.x

Short Report

Lysosomal multienzymatic complex-related diseases: a genetic study among Portuguese patients

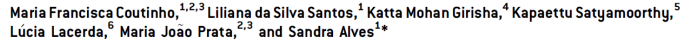
Coutinho MF, Lacerda L, Macedo-Ribeiro S, Baptista E, Ribeiro H, Prata MJ, Alves S. Lysosomal multienzymatic complex-related diseases: a genetic study among Portuguese patients.
Clin Genet 2011. © John Wiley & Sons A/S, 2011

MF Coutinho[a], L Lacerda[a], S Macedo-Ribeiro[b], E Baptista[a], H Ribeiro[a], MJ Prata[c,d] and S Alves[a]

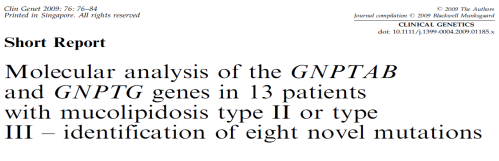
Mucolipidosis type II and III

. A set of 23 unrelated ML II and III cases from several origins was screened:
18 different mutations → 16 in the *GNPTG* gene and 2 in the *GNPTG* gene.
Of those, 13 were novel: 11 in the *GNPTAB* gene and 2 in the *GNPTG* gene.

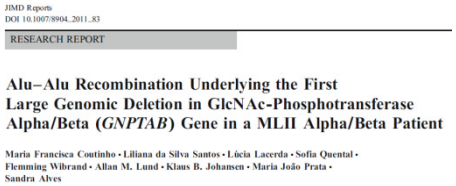
• Identification of the first large deletion in the *GNPTAB gene*


Mucolipidosis Type II α/β With a Homozygous Missense Mutation in the *GNPTAB* Gene

Maria Francisca Coutinho,[1,2,3] Liliana da Silva Santos,[1] Katta Mohan Girisha,[4] Kapaettu Satyamoorthy,[5] Lúcia Lacerda,[6] Maria João Prata,[2,3] and Sandra Alves[1]*

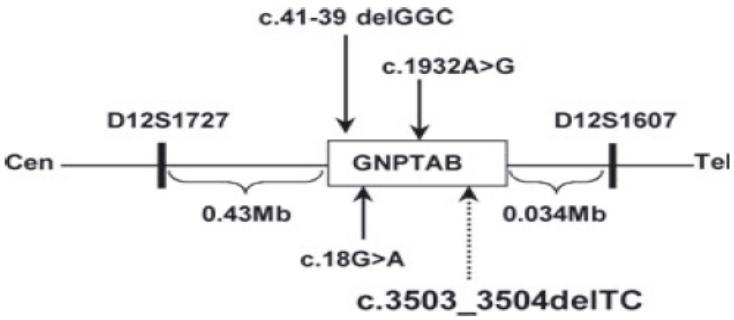

Short Report

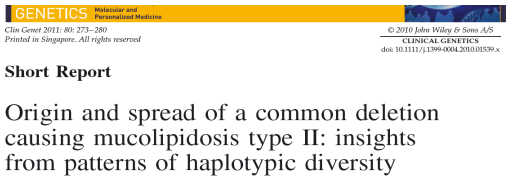
Molecular analysis of the *GNPTAB* and *GNPTG* genes in 13 patients with mucolipidosis type II or type III – identification of eight novel mutations


RESEARCH REPORT

Alu–Alu Recombination Underlying the First Large Genomic Deletion in GlcNAc-Phosphotransferase Alpha/Beta (*GNPTAB*) Gene in a MLII Alpha/Beta Patient

Origin and spread of a common deletion c.3503_3504delTC




Short Report

Origin and spread of a common deletion causing mucolipidosis type II: insights from patterns of haplotypic diversity

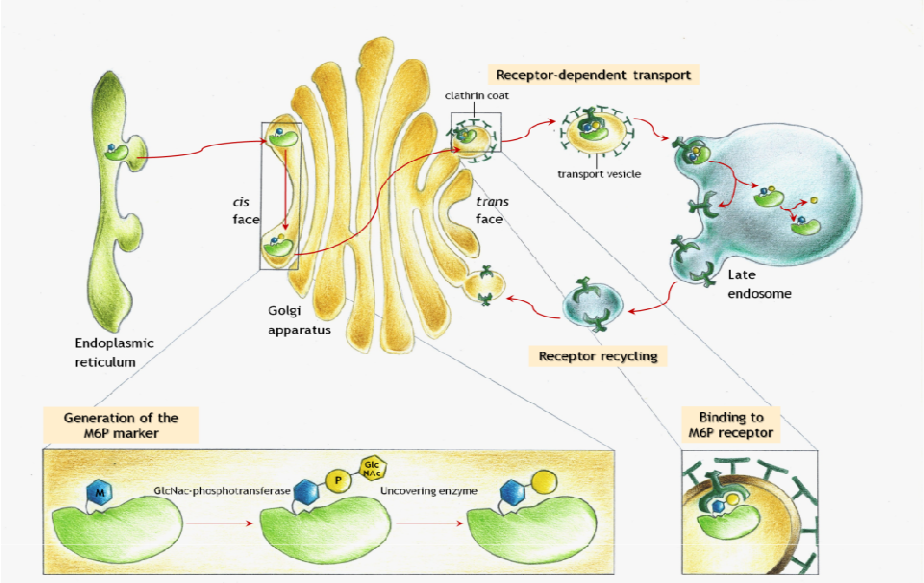
44 patients and 16 carriers from different geographic regions were analyzed for

3 intragenic polymorphisms and
2 microsatellite markers flanking the *GNPTAB* gene.

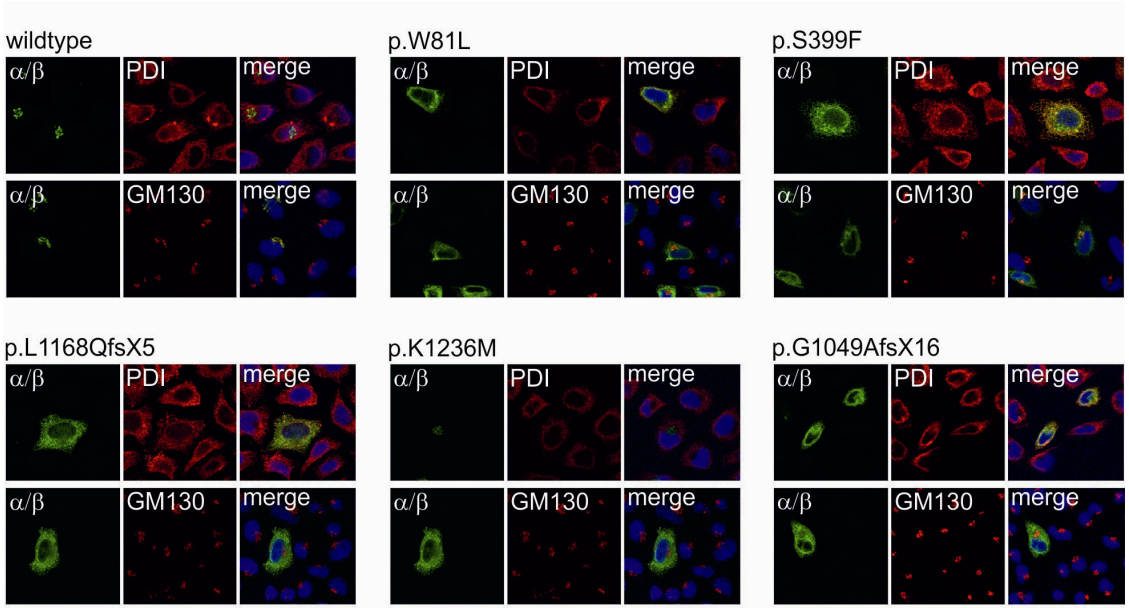
A common haplotype was identified in all chromososmes bearing the deletion: common origin (aprox. 2000 years ago)

Its geographical distribution also suggested it to have arisen in the peri-Mediterranean region.

Molecular and cellular characterization of lysosomal diseases: diversity, frequency and causality



Missense and frameshift mutations that are associated with a severe clinical phenotype cause retention of the encoded protein in the endoplasmic reticulum in its precursor form



RESEARCH ARTICLE

Human Mutation

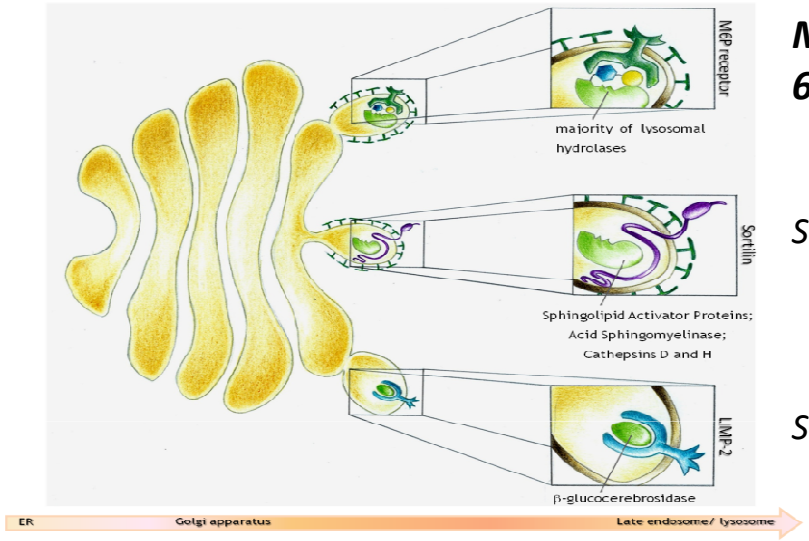
OFFICIAL JOURNAL HGVS HUMAN GENOME VARIATION SOCIETY www.hgvs.org

Mucolipidosis II-Related Mutations Inhibit the Exit from the Endoplasmic Reticulum and Proteolytic Cleavage of GlcNAc-1-Phosphotransferase Precursor Protein (*GNPTAB*)

Raffaella De Pace,[1†] Maria Francisca Coutinho,[2,3,4†] Friedrich Koch-Nolte,[5] Friedrich Haag,[5] Maria João Prata,[3,4] Sandra Alves,[2] Thomas Braulke,[1] and Sandra Pohl[1*]

Molecular and biochemical characterization of LSDs patients and pathophysiological mechanisms

LSD patients without specific diagnosis

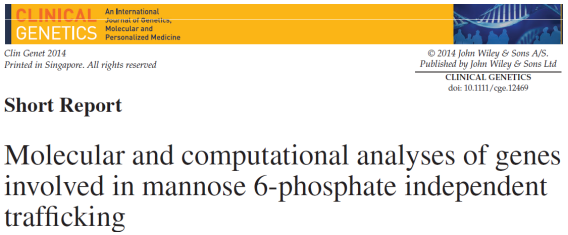


Molecular screening of genes involved in mannose 6-phosphate independent trafficking

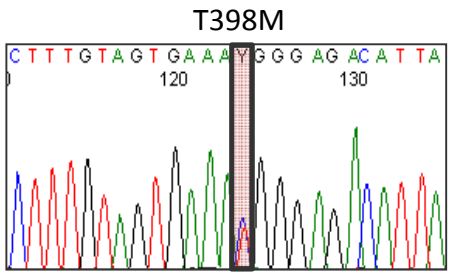
SORT1

SCARB2

120 individuals with clinical suspicion of LSD but without definitive biochemical and/or molecular diagnosis,
no novel mutations were detected either on the *SCARB2* or on the *SORT1* genes.


CLINICAL GENETICS
An International Journal of Genetics, Molecular and Personalized Medicine
Clin Genet 2014
Printed in Singapore. All rights reserved
© 2014 John Wiley & Sons A/S.
Published by John Wiley & Sons Ltd
CLINICAL GENETICS
doi: 10.1111/cge.12469
Short Report
Molecular and computational analyses of genes involved in mannose 6-phosphate independent trafficking

SCARB2 mutations as phenotypic modifiers in Gaucher disease



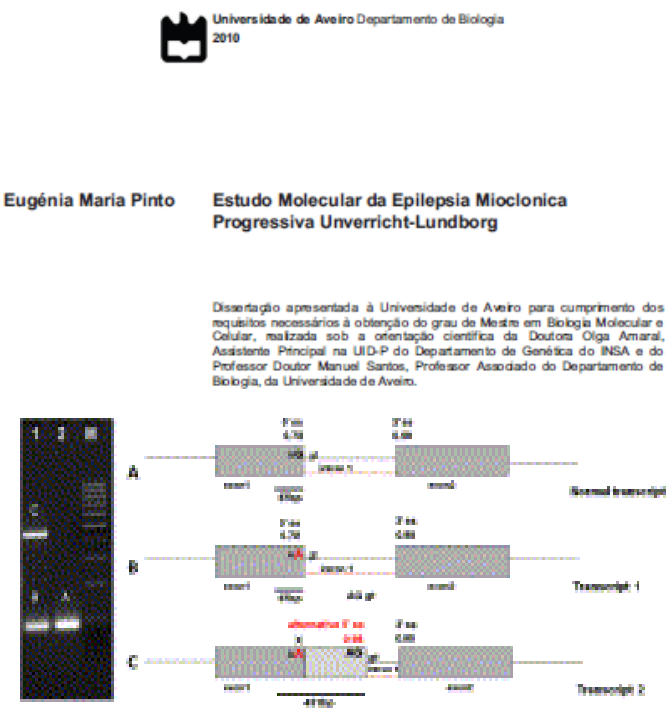
To evaluate the role of *SCARB2* mutations on the Gaucher Disease phenotype, the whole cohort of Portuguese GD patients (which totalizes 91 individuals) was screened and 1 novel mutation in the *SCARB2* gene identified.

Coutinho MF (2014) under preparation

Unverricht- Lundborg

Unverricht- Lundborg disease (ULD ou EPM1, MIM 254800), is a myoclonic epilepsy, caused by mutations in the *CSTB* gene which lead to impaired function of cystatin B and failure in the inhibition of lysosomal proteases.

•Identification of a **rare molecular mechanism** causal of Unverricht Lundborg disease in a unique Portuguese patient.

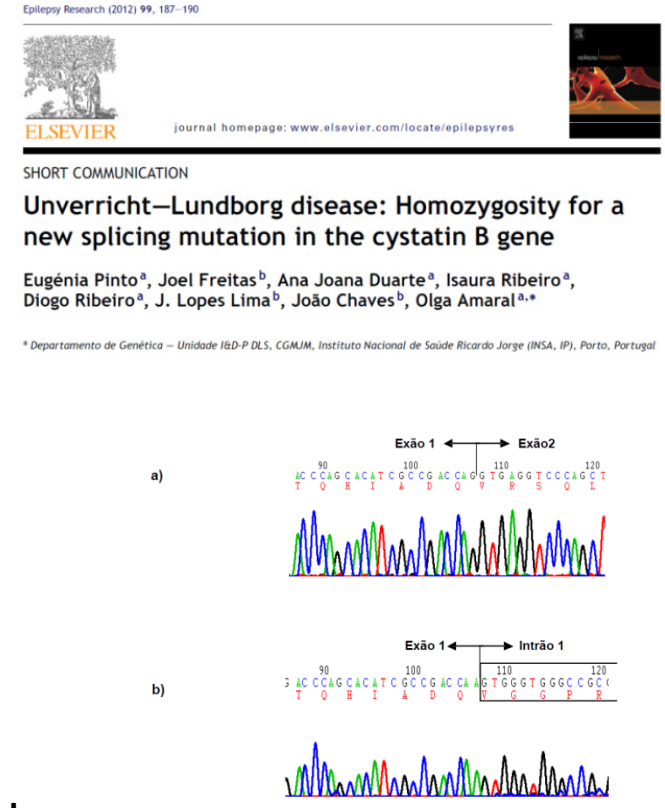


Universidade de Aveiro Departamento de Biologia
2010

Eugénia Maria Pinto

Estudo Molecular da Epilepsia Mioclonica Progressiva Unverricht-Lundborg

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biologia Molecular e Celular, realizada sob a orientação científica da Doutora Olga Amaral, Assistente Principal na UID-P do Departamento de Genética do INSA e do Professor Doutor Manuel Santos, Professor Associado do Departamento de Biologia, da Universidade de Aveiro.



Epilepsy Research (2012) 99, 187–190

ELSEVIER journal homepage: www.elsevier.com/locate/epilepsyres

SHORT COMMUNICATION

Unverricht—Lundborg disease: Homozygosity for a new splicing mutation in the cystatin B gene

Eugénia Pinto[a], Joel Freitas[b], Ana Joana Duarte[a], Isaura Ribeiro[a], Diogo Ribeiro[a], J. Lopes Lima[b], João Chaves[b], Olga Amaral[a,*]

[a] Departamento de Genética – Unidade I&D-P DLS, CGMJM, Instituto Nacional de Saúde Ricardo Jorge (INSA, IP), Porto, Portugal

Project FCT: PIC/IC/82822/2007 (2010-2013), IR: Olga Amaral

Molecular and cellular characterization of normal and mutant cystatin B

Unverricht-Lundborg disease is caused by mutations in the cystatin B gene compromising its protective anti-protease function. The normal protein has several cellular localizations, it is found in the nucleus, cytosol and lysosome.

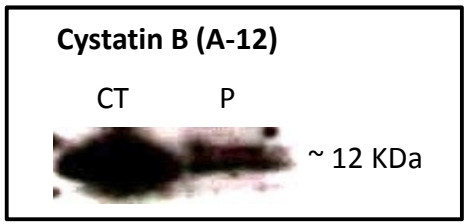


Fig 1: Western analysis of skin fibroblasts of control and patient

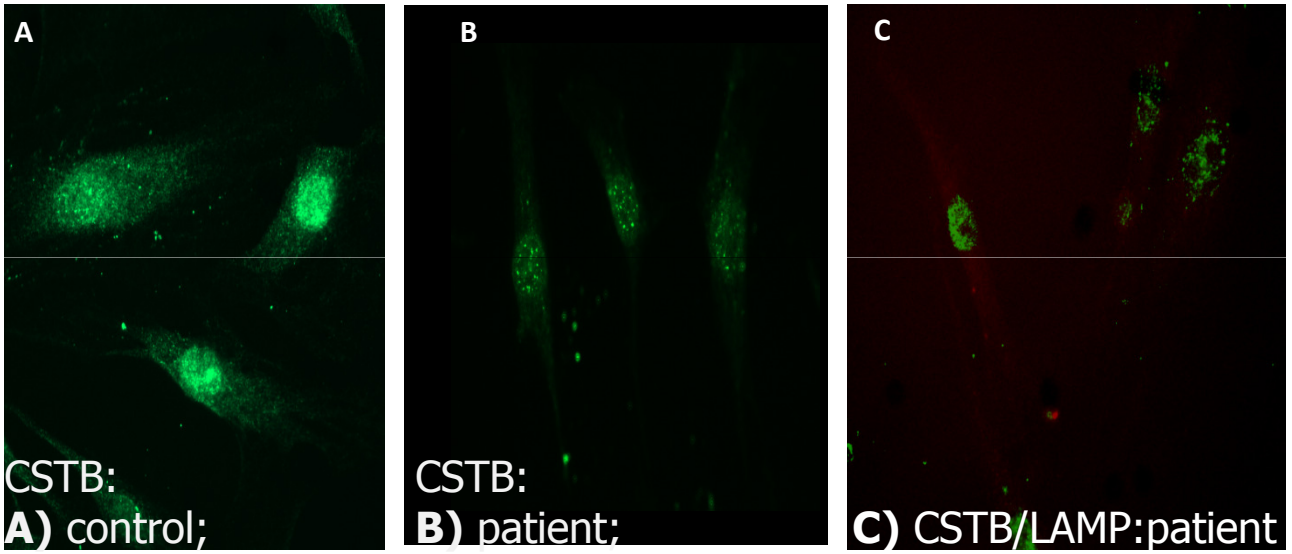


Fig 2: IF study in skin fibroblasts with specific antibodies

- What is the pathogenic mechanism involved in this progressive epilepsy?
- What is the extent of lysosomal involvement?
- In patient fibroblasts the protein quantity is clearly reduced (Fig.1 and Fig.2) although its location seems to be preserved. Could there be differences in other cell types?

Project FCT: PIC/IC/82822/2007 (2010-2013), IR: Olga Amaral

Caracterization of the *IDUA* gene mutation W402X (Diogo Ribeiro, MSc)

Universidade do Minho
Escola de Ciências da Saúde

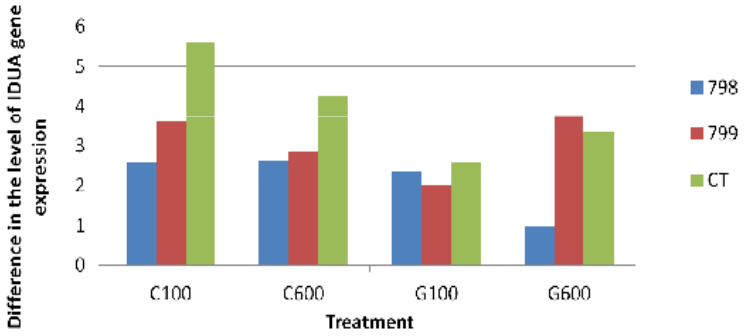
Diogo Alexandre do Nascimento Ribeiro

Study of the W402X mutation: frequency of the lysosomal α-L-iduronidase variant in the Portuguese population and function analysis

Estudo da mutação W402X: frequência desta variante da α-L-iduronidase lisossomal na população portuguesa e análise da sua função

Mucopolysaccharidosis type I (MPS I) results from the defective activity of the lysosomal α-L-iduronidase enzyme (IDUA; EC 3.2.1.76). MPS I is one of the more frequent MPSs. In the Portuguese population it is often associated with mutation W402X (over 60% in unrelated patients).

- Mutation W402X results in NMD, seems amenable to correction by nonsense suppression.
- Presence of different haplotypes in Portugal



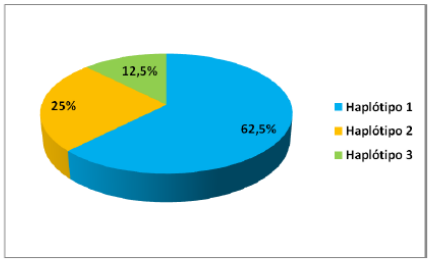
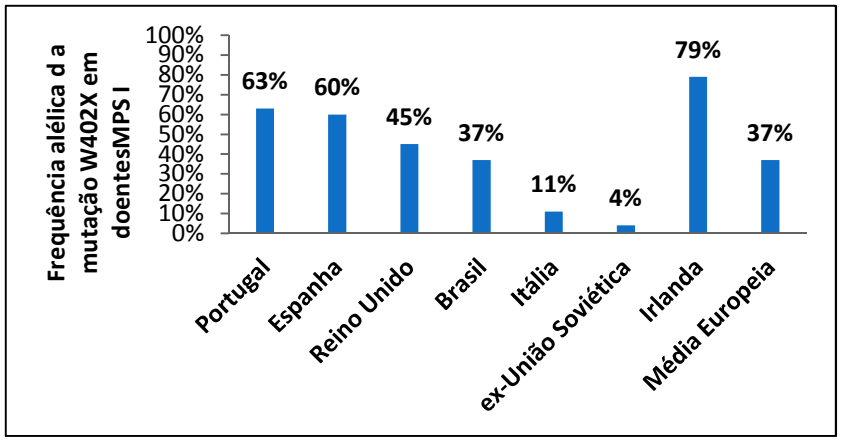
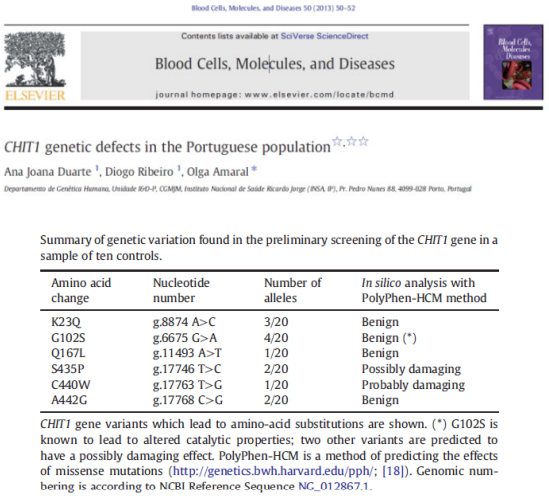


Figura 9 – Gráfico com as percentagens obtidas dos diferentes haplótipos encontrados nos portadores da mutação W402X.



Project FCT: PIC/IC/82822/2007 (2010-2013), IR: Olga Amaral

Molecular and cellular characterization of lysosomal diseases: diversity, frequency and causality



Blood Cells, Molecules, and Diseases 50 (2013) 50–52

Contents lists available at SciVerse ScienceDirect

Blood Cells, Molecules, and Diseases

journal homepage: www.elsevier.com/locate/bcmd

CHIT1 genetic defects in the Portuguese population☆,☆☆

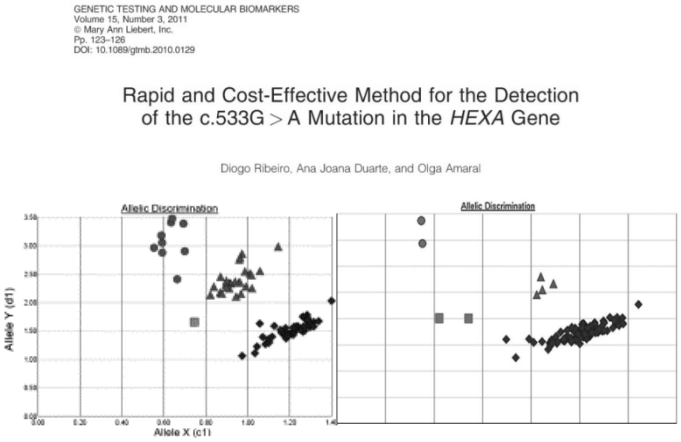
Ana Joana Duarte [1], Diogo Ribeiro [1], Olga Amaral *

Departamento de Genética Humana, Unidade Id-D-P, CGMJM, Instituto Nacional de Saúde Ricardo Jorge (INSA, IP), Pr. Pedro Nunes 88, 4099-028 Porto, Portugal

Summary of genetic variation found in the preliminary screening of the *CHIT1* gene in a sample of ten controls.

Amino acid change	Nucleotide number	Number of alleles	*In silico* analysis with PolyPhen-HCM method
K23Q	g.8874 A>C	3/20	Benign
G102S	g.6675 G>A	4/20	Benign (*)
Q167L	g.11493 A>T	1/20	Benign
S435P	g.17746 T>C	2/20	Possibly damaging
C440W	g.17763 T>G	1/20	Probably damaging
A442G	g.17768 C>G	2/20	Benign

CHIT1 gene variants which lead to amino-acid substitutions are shown. (*) G102S is known to lead to altered catalytic properties; two other variants are predicted to have a possibly damaging effect. PolyPhen-HCM is a method of predicting the effects of missense mutations (http://genetics.bwh.harvard.edu/pph/; [18]). Genomic numbering is according to NCBI Reference Sequence NC_012867.1.



GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 15, Number 3, 2011
© Mary Ann Liebert, Inc.
Pp. 123–126
DOI: 10.1089/gtmb.2010.0129

Rapid and Cost-Effective Method for the Detection of the c.533G > A Mutation in the *HEXA* Gene

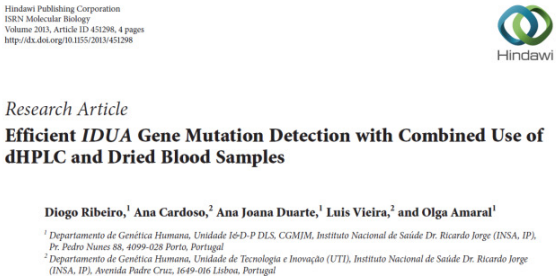
Diogo Ribeiro, Ana Joana Duarte, and Olga Amaral

Allelic Discrimination

Allele Y (d1)

Allele X (c1)

Allelic Discrimination



Hindawi Publishing Corporation
ISRN Molecular Biology
Volume 2013, Article ID 451298, 4 pages
http://dx.doi.org/10.1155/2013/451298

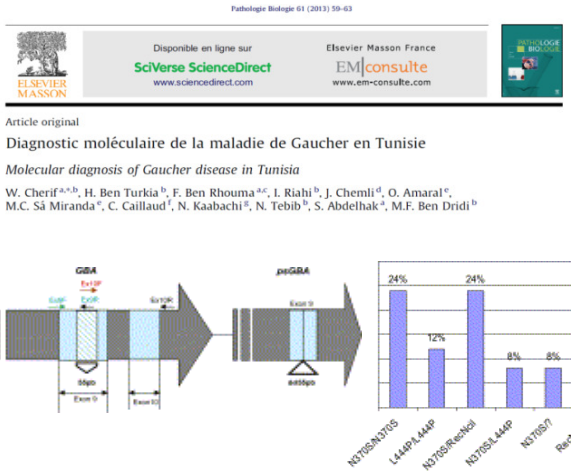
Hindawi

Research Article

Efficient *IDUA* Gene Mutation Detection with Combined Use of dHPLC and Dried Blood Samples

Diogo Ribeiro,[1] Ana Cardoso,[2] Ana Joana Duarte,[1] Luis Vieira,[2] and Olga Amaral[1]

[1] Departamento de Genética Humana, Unidade Id-D-P DLS, CGMJM, Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA, IP), Pr. Pedro Nunes 88, 4099-028 Porto, Portugal
[2] Departamento de Genética Humana, Unidade de Tecnologia e Inovação (UTI), Instituto Nacional de Saúde Dr. Ricardo Jorge (INSA, IP), Avenida Padre Cruz, 1649-016 Lisboa, Portugal



Pathologie Biologie 61 (2013) 59–63

Disponible en ligne sur SciVerse ScienceDirect www.sciencedirect.com

Elsevier Masson France EM|consulte www.em-consulte.com

Article original

Diagnostic moléculaire de la maladie de Gaucher en Tunisie

Molecular diagnosis of Gaucher disease in Tunisia

W. Cherif [a,*,b], H. Ben Turkia [b], F. Ben Rhouma [a,c], I. Riahi [b], J. Chemli [d], O. Amaral [e], M.C. Sá Miranda [e], C. Caillaud [f], N. Kaabachi [g], N. Tebib [b], S. Abdelhak [a], M.F. Ben Dridi [b]

- Development of **low cost mutation detection** techniques (Diogo Ribeiro et al. 2011; 2013)
- Identification of **rare variants** which influence prognosis (Ana Duarte et al. 2013; Amaral et al. 2013; Rodrigues et al, 2004)
- Clarifying **molecular basis of Lysosomal Diseases** (Amaral, et al. 1997, 1999 & 2000; Ribeiro, et al. 2001; Pinto et al. 2010)
- **Prevalence** of various disease causing mutations in different populations (Amaral, et al. 1994; Marcao, et al. 1999; Pinto et al. 2004; Lugowska et al. 2005; Cherif et al. 2009 & 2013)

Project FCT: PIC/IC/82822/2007 (2010-2013), IR: Olga Amaral

Molecular and cellular characterization of lysosomal diseases: diversity, frequency and causality

Future plans

Use of next-generation-sequencing (NGS) as an approach for the diagnosis of LSDs

Aim

To develop a next generation sequencing (NGS) based workflow for the identification of exon-variations in a group of genes involved in lysosomal function.

Target

Uncharacterized patients presenting symptoms associated with LSDs

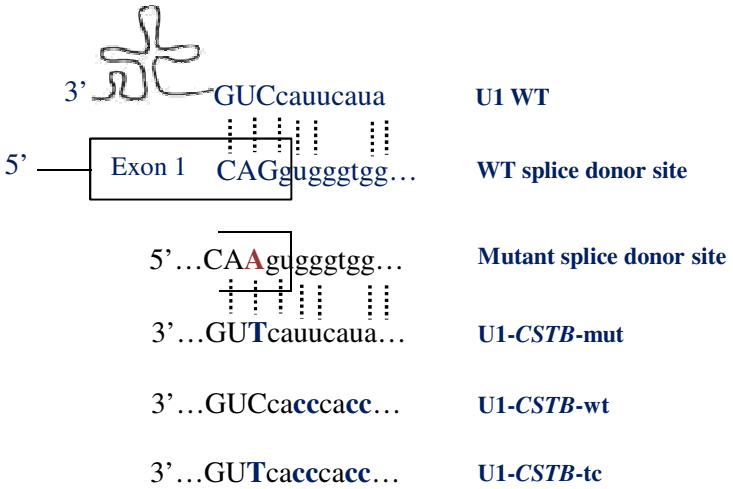
The use of next generation sequencing in this group of patients with suspected LSD but no definitive diagnosis would be the ideal approach to search for the genetic basis of disease in these individuals

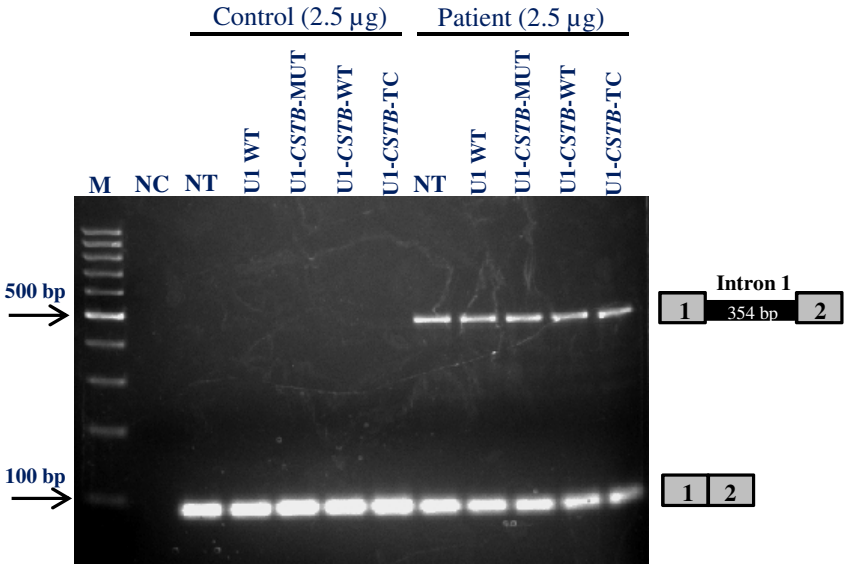
In the scope of Comissão Coordenadora do Tratamento das Doenças Lisossomais de Sobrecarga (CCTDLS) that operates under INSA coordination - Despacho n.º 2545/2013

Splicing Therapeutics - antisense oligonucleotide and U1snRNA mediated therapeutic strategies

Unverricht- Lundborg disease - c.66G>A (*CSTB gene)*

U1 snRNA-mediated Gene Therapeutic Approach in ULD patient-derived fibroblasts.





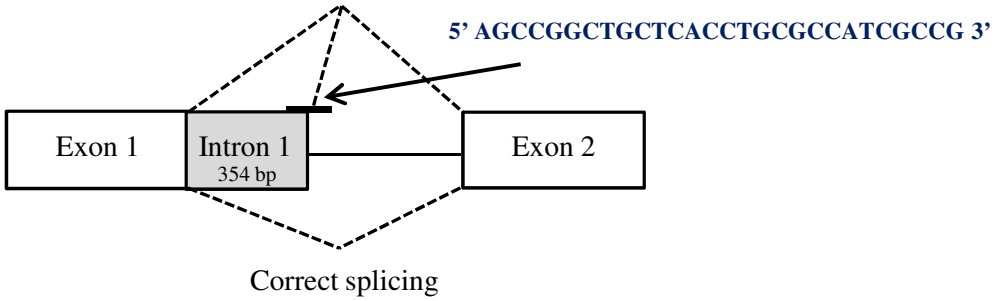
No correction

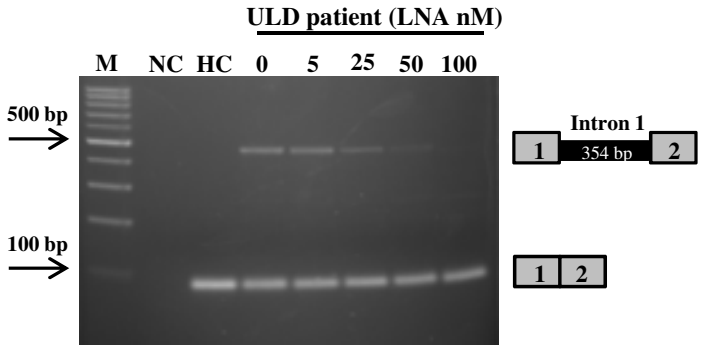
Matos et al. under preparation

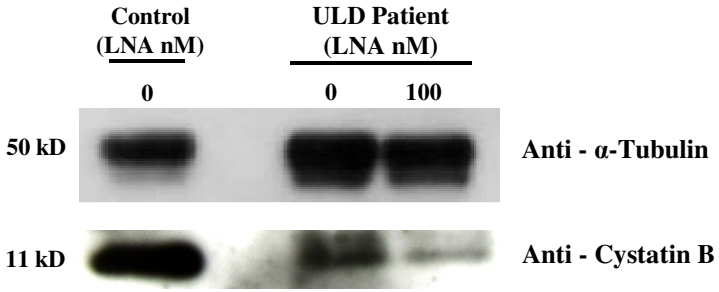
Splicing Therapeutics - antisense oligonucleotide and U1snRNA mediated therapeutic strategies

Unverricht- Lundborg disease - c.66G>A (*CSTB gene)*

Antisense Oligonucleotide Therapeutic Approach in ULD patient-derived fibroblasts.







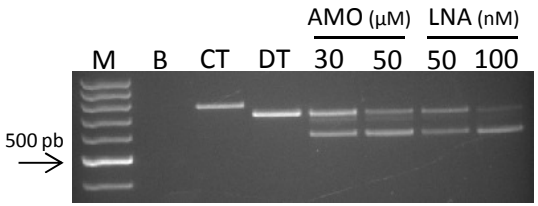
Successful correction of the aberrant transcript

Matos et al. under preparation

Splicing Therapeutics - Antisense oligonucleotide and U1snRNA mediated therapeutic strategies

Mucopolysaccharidosis type II c.1122C>T (*IDS* gene)

Antisense Oligonucleotides treatment of patient's fibroblasts



No correction

Development of antisense therapeutic approaches remains a challenge in genes under fine regulation mechanisms as is the case of *IDS* gene.

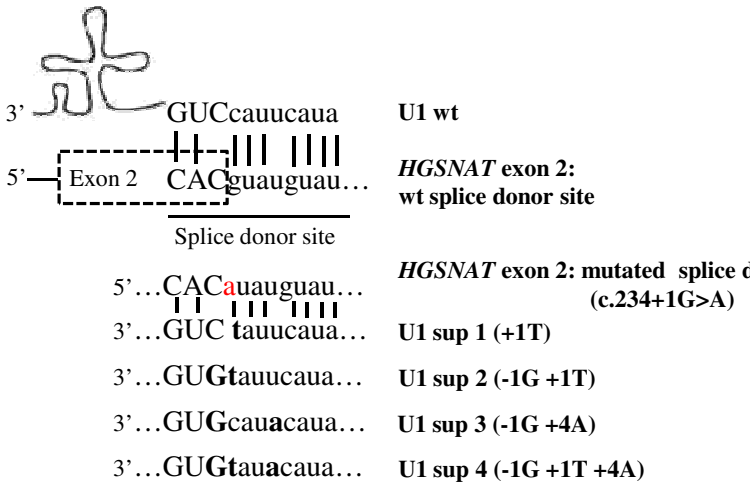
Matos et al. under preparation

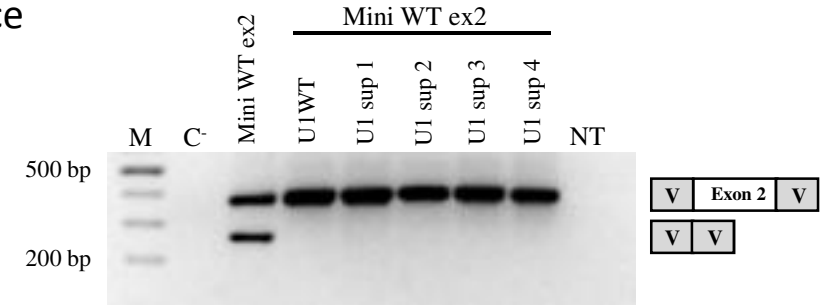
Splicing Therapeutics - Antisense oligonucleotide and U1snRNA mediated therapeutic strategies

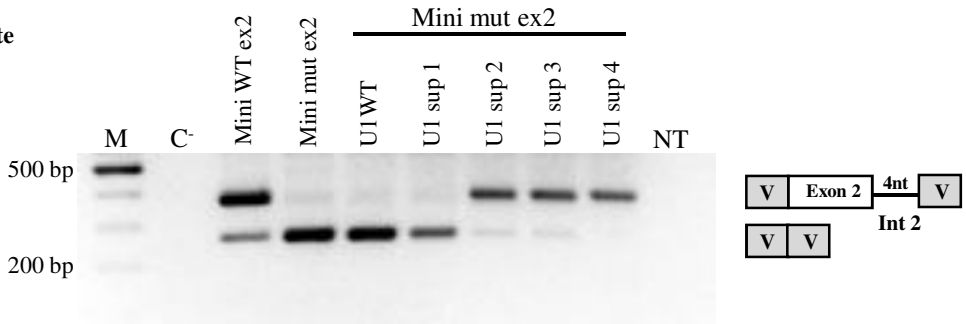
Mucopolysaccharidosis type IIIC c.234+1G>A (*HGSNAT gene)*

U1 snRNA-mediated Gene Therapeutic Approach in MPS IIIC patient-derived fibroblasts.

A **partial recovery** was achieved with a modified U1 snRNA that completely matches the splice donor site.







Matos et al. submitted

Development of innovative therapies for LSDs

Splicing Therapeutics - Antisense oligonucleotide and U1snRNA mediated therapeutic strategies

Mutations under study:

Mucopolysaccharidosis I: c.1650+5G>A (*IDUA* gene)

Mucolipidosis III: c.3335+6T>G (*GNPTAB gene)*

3 U1 variants were constructed for c.1650+5G>A

2 U1 mutant vectors were constructed c.3335+6T>G

transfected using Lipofectamine LTX

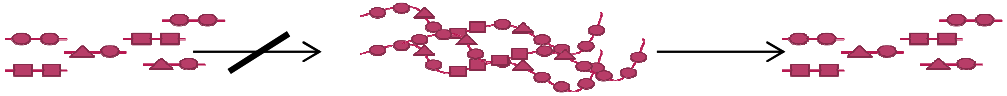
No correction

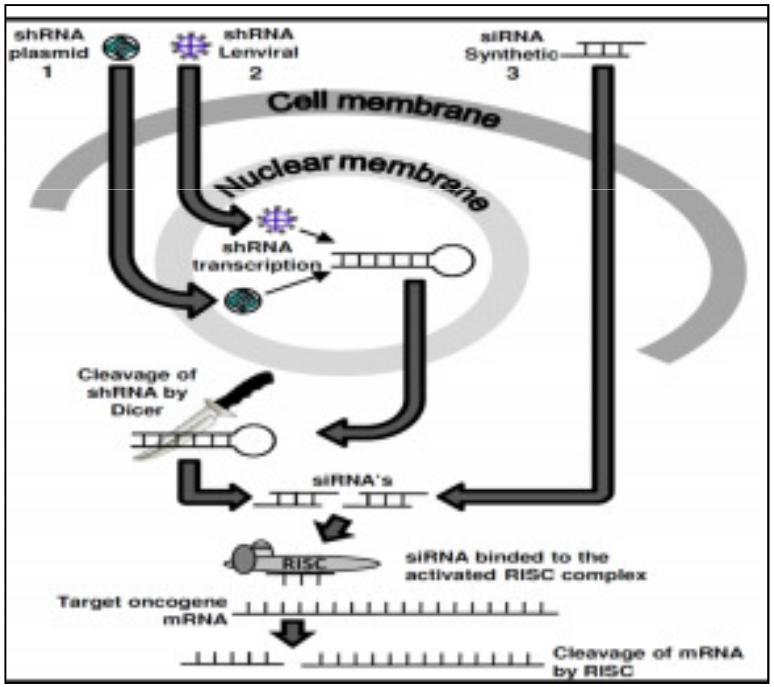
Novel approach: U1 lentivirus plasmid will be used to generate through mutagenesis the same U1 mutant vectors that will be used in a model cell line with mutation-disease minigenes as well as directly in patient's fibroblasts through the transduction lentiviral technique.

Substrate Reduction Therapy for Mucopolysaccharidoses through RNAi

RNAi-dependent strategy based

Selective downregulation of genes involved in the biosynthesis of the glycosaminoglycans (GAGs) that accumulate in patients suffering from Mucopolysaccharidoses.

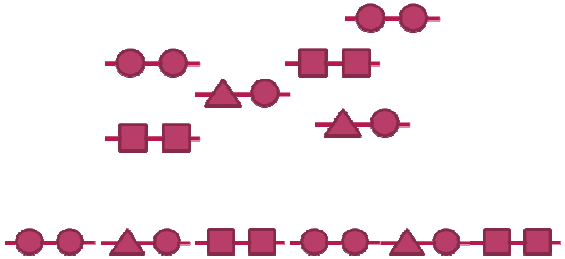




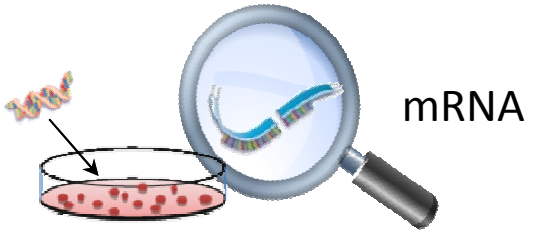
Substrate Reduction Therapy for Mucopolysaccharidoses through RNAi

WorkPlan

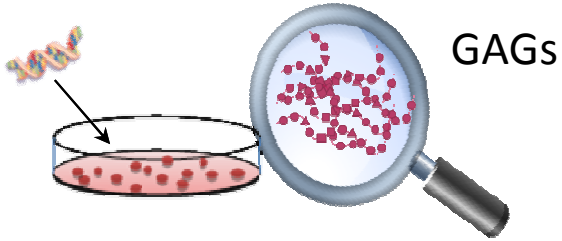
- Design specific siRNAs targeting genes involved in the biosynthesis of GAGs.
  - Targets:
    - *CHSY1*, *CHPF* e *CHSY3;*
    - *EXTL2* e *EXTL3;*
    - *XYLT1*,*XYLT2*,*GALTI*, e *GALTII*



- Evaluate the effect of each specific siRNA in the mRNA expression levels and in its target protein expression levels.



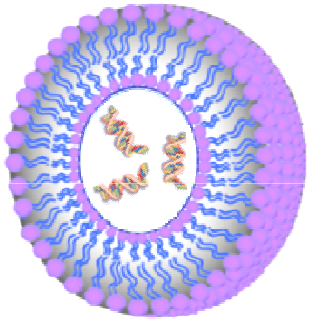
- Evaluate the effect of each specific siRNA and/or combination of individual siRNAs on the intralysosomal accumulation of GAGs in MPS patients' cell lines.



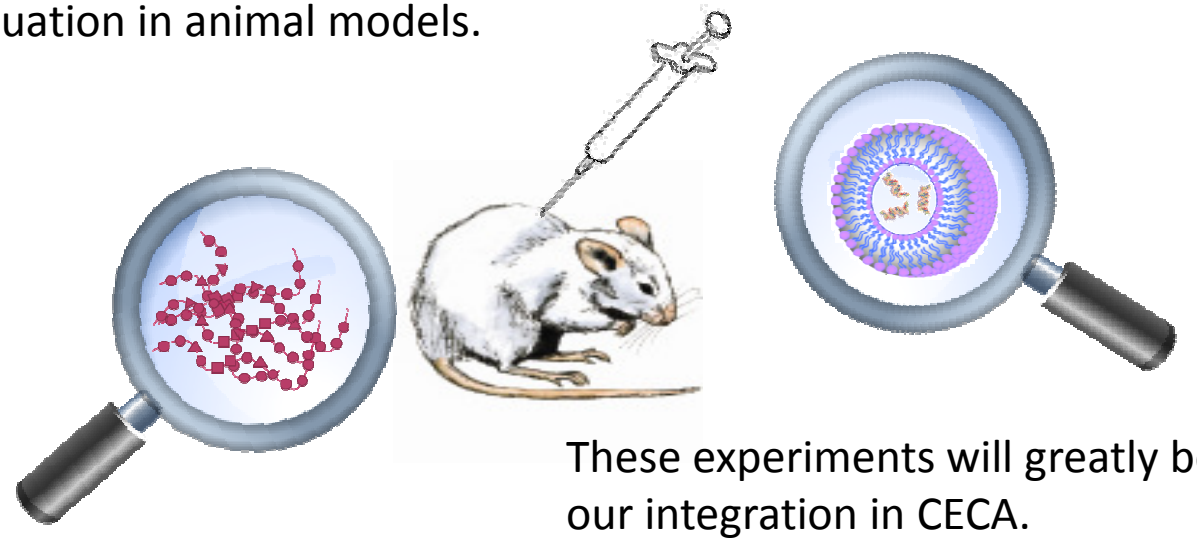
Development of innovative therapies for LSDs

Future directions...

- Design a proper siRNA delivery strategy for therapeutic purposes

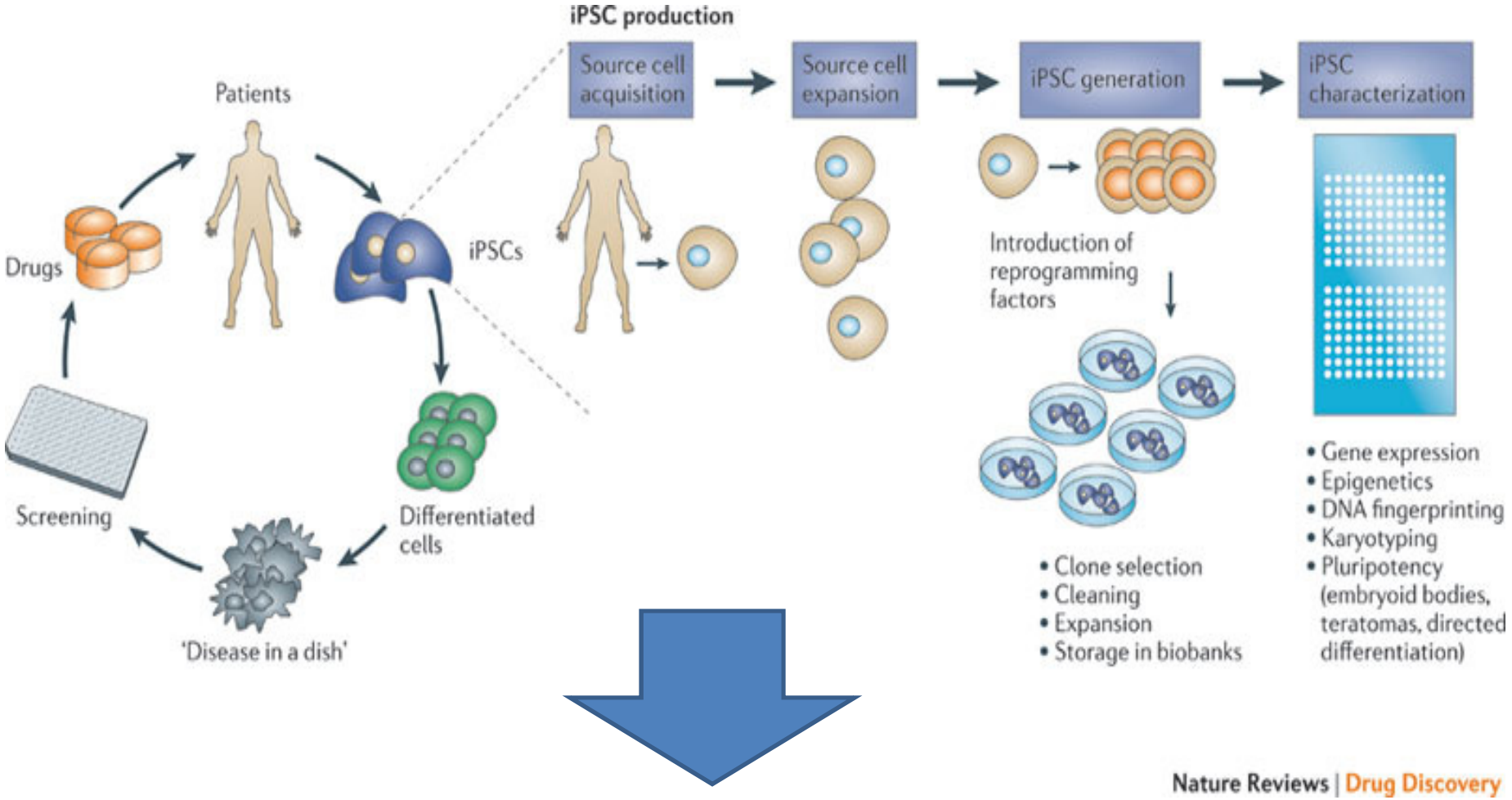


- *In vivo* evaluation in animal models.



These experiments will greatly benefit with our integration in CECA.

Future directions: generation of iPSCells as disease specific cellular models for LSDs



Figures: Grskovic et al, 2011, Nat Rev Drug Discovery, 10:915-929

Generation of disease specific cell-targets

CECA might provide a favorable environment for fostering part of this work

Future directions : iPSCs as disease specific cellular models for LSDs

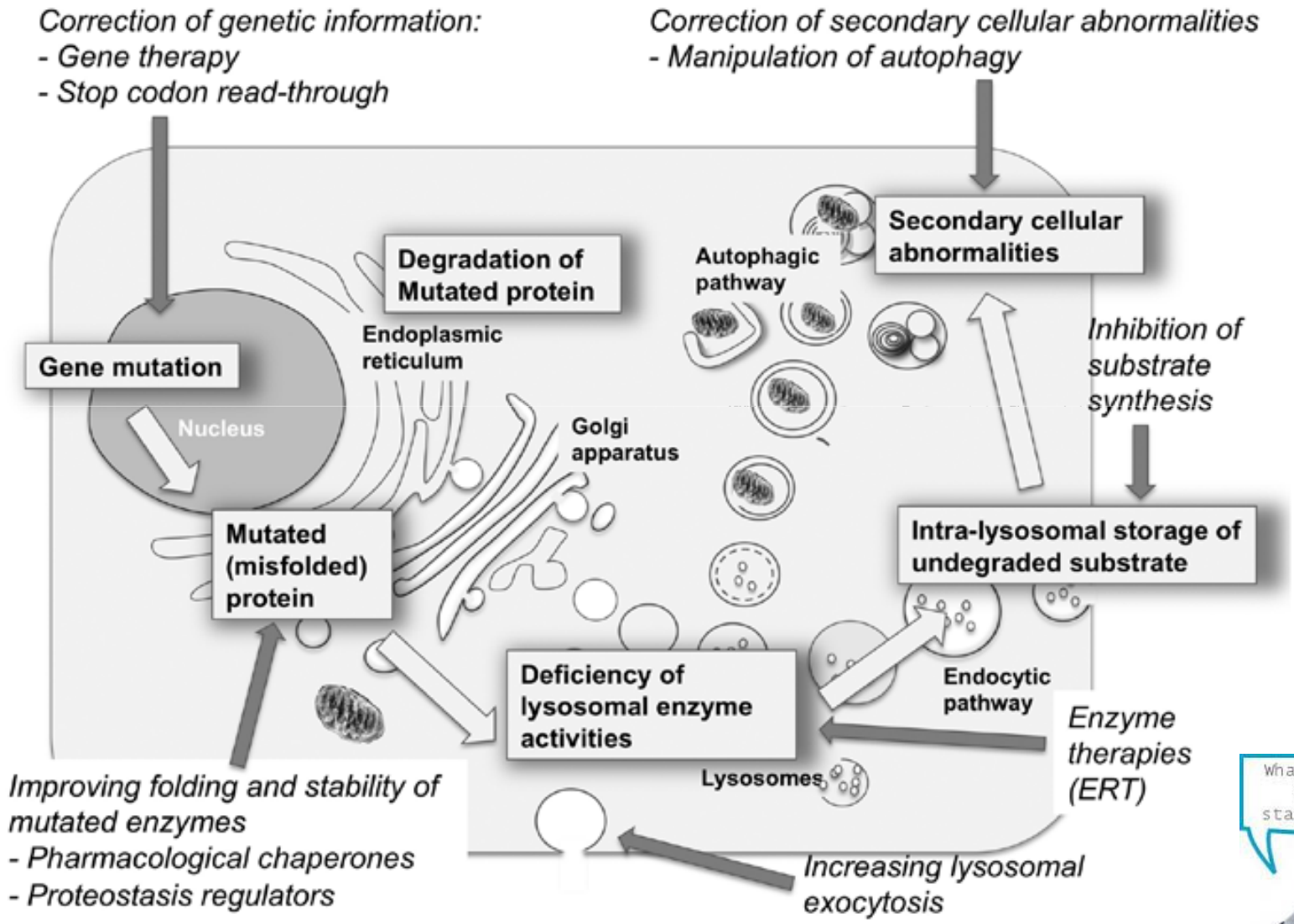


Figure from: Parenti et al, 2013, International J Molecular Med, 31(1), 11-20