18th Hungarian MPS Conference

Gödöllő-Máriabesnyő, September 14-16, 2012

Morquio B Disease (MBD)

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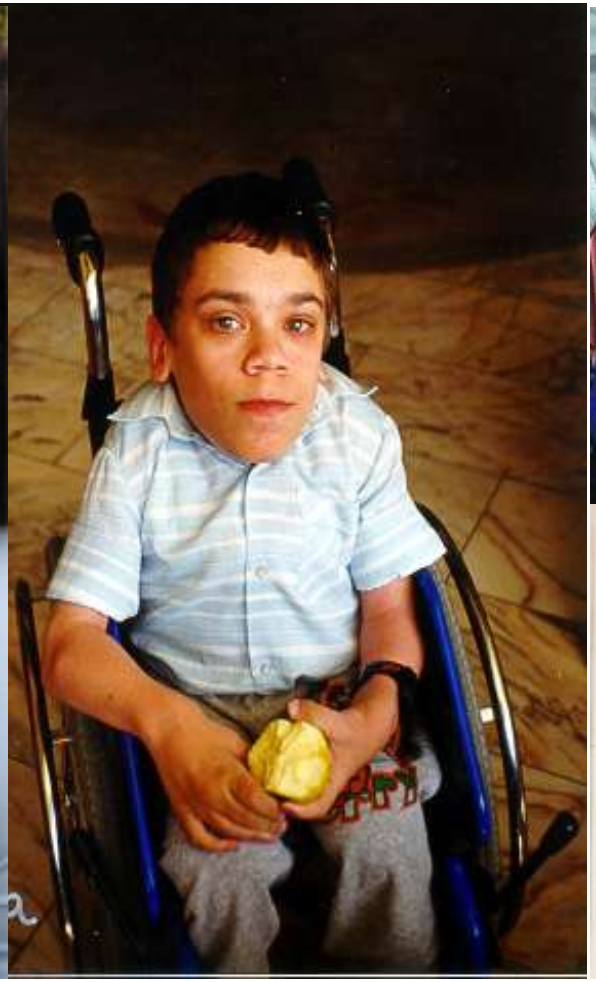
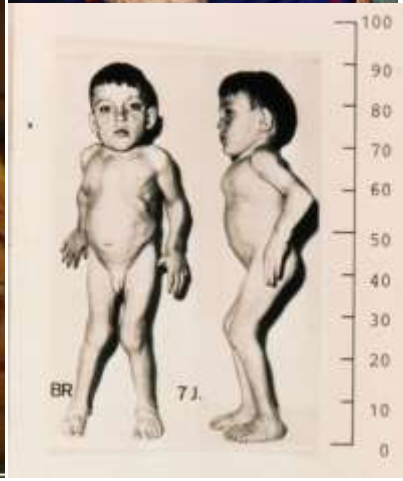
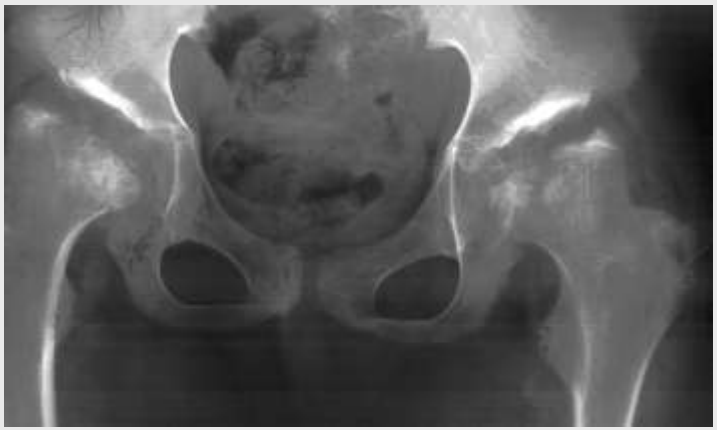
Morquio Disease



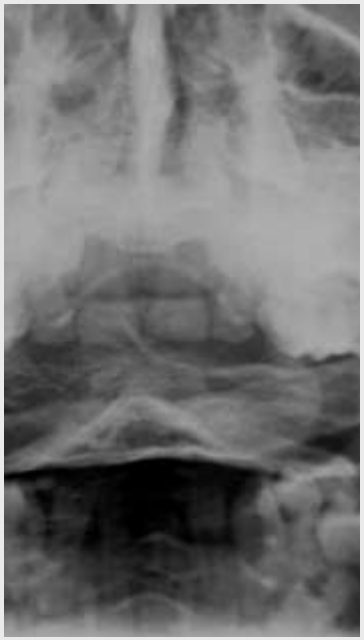


Morquio disease-

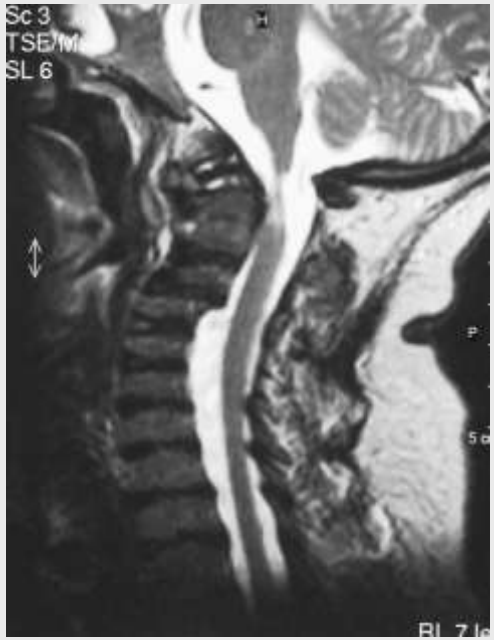
dysostosis multiplex & normal CNS function



dysplasia of hip joints



hypoplasia of dens axis



cervical myelocompression

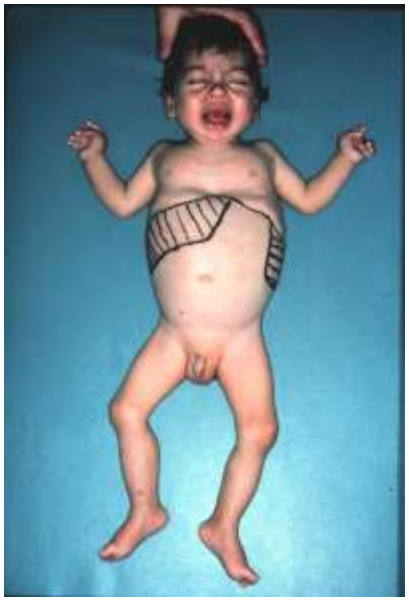
Morquio disease and GM1-Gangliosidosis

Morquio disease					
Type	**Bones**	**CNS**	**storage**	**Gene (Enzyme)**	**Incidence**
Type A	+++	norm.	Keratan sulfate	GALNS (GalNAc-6-sulfatase)	<1: 250.000
Type B	++	norm.	Keratan sulfate, oligosaccharides	GLB1 (ß-Galactosidase)	unknown

GM1- Gangliosidosis					
Type	**Bones**	**CNS**	**storage**	**Gene (Enzyme)**	**Incidence**
infantile juvenile adult	++ norm. to ++ norm to ++	+++ ++ ++	oligosaccharides, GM1-ganglioside, keratan sulfate	GLB1 (ß-Galactosidase)	1:100,000-1:200,000

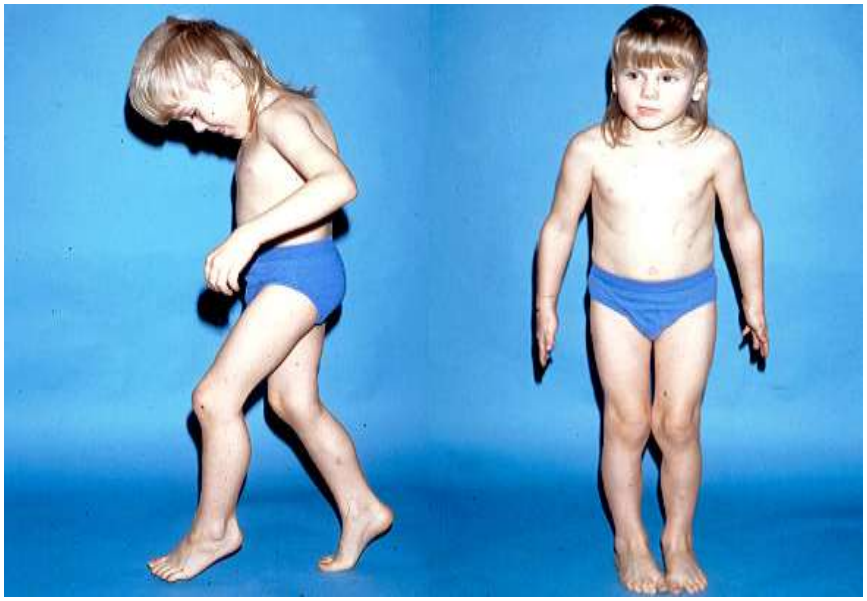
Disease Types

caused by deficiency of acid ß-Galactosidase



GM1-Gangliosidosis, Type 1
6 months of age

Neurodegeneration,
cherry red spots,
Hepatosplenomegaly,
skeletal changes,



GM1- Gangliosidosis, Type 3
7 years of age

mental impairment
minor skeletal changes

Morquio B disease
19 years of age

mentally normal
skeletal changes

Morquio B disease (MBD)

in Austria

Patient 6,
5 years

Patient 3,
19 years

Patient 2,
52 years

How can alterations in a single gene cause

these two distinctly different diseases ?

Morquio B disease in Austria

6 cases origin from a small region in Southern Austria

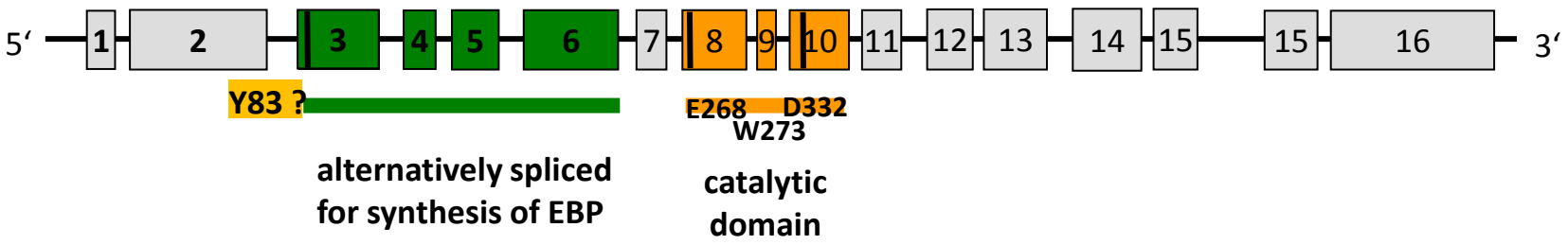
The Genotypes of MBD Cases

Paschke et al.
Hum Genet (2001)
109: 159-166

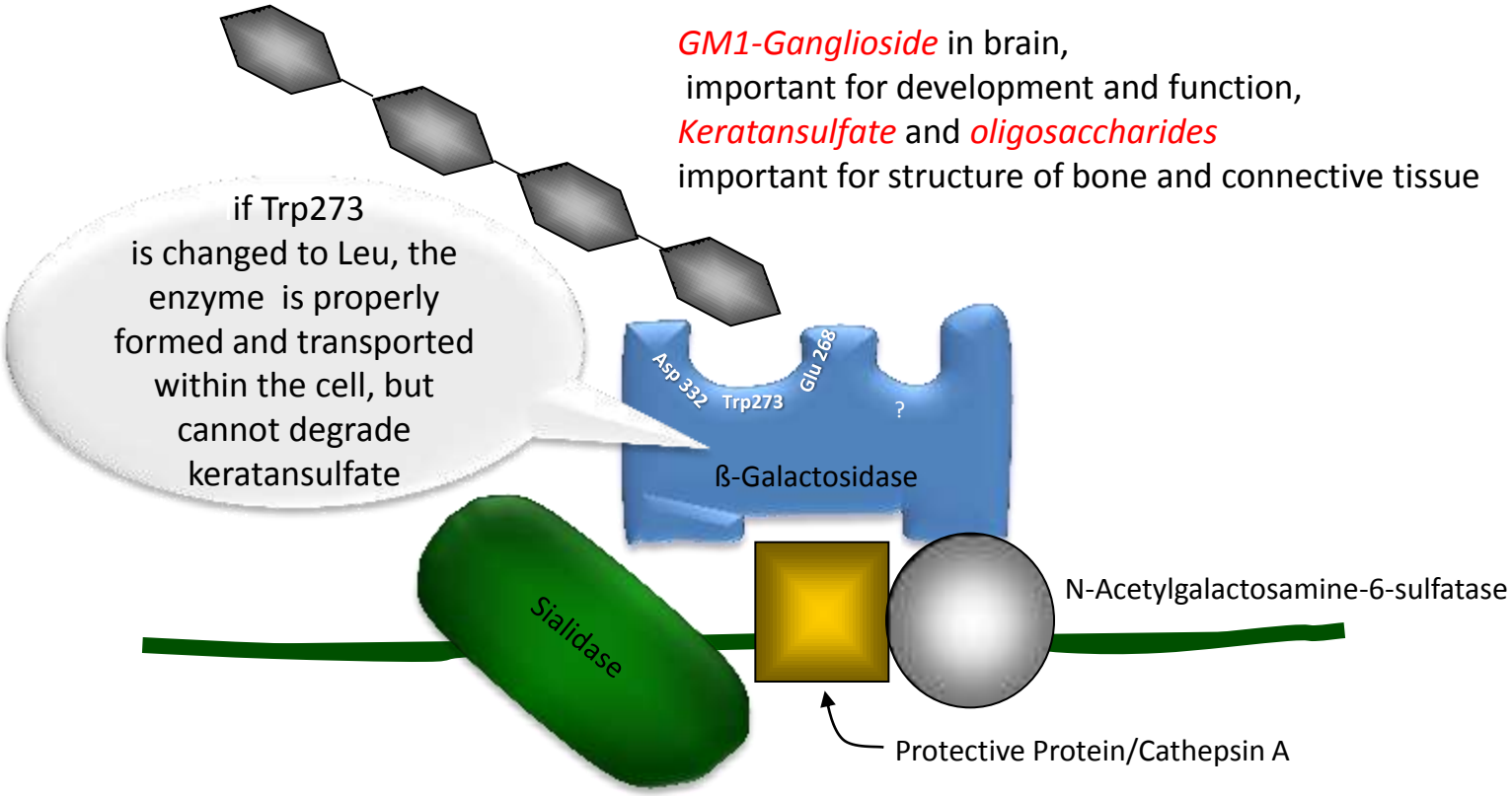
Nr.	Code	Genotype	Exon	Age	Onset	Neur	Bone	Origin
1	R.E.	W273L/W273L	VIII	43	5	none	+++	Austrian
2	B.M.	W273L/W273L	VIII	52	5	none	+++	Austrian
3	E.M.	W273L/W273L	VIII	21	4	none	+++	Austrian
4	A.M.	W273L/W273L	VIII	21	3	none	+++	Austrian
5	H.F.	W273L/W273L	VIII	43	?	none	+++	Austrian
6	St.M.	W273L/W273L	VIII	5	3	none	+++	Austrian
7	90RD26	W273L/W273L	VIII	21	7	none	+++	French
8	94RD37	W273L/W273L	VIII	29	3	none	+++	Dutch
9	P.S.	W273L/W273L	VIII	18	?	none	+++	Bosnian
10	Pl	W273L/W273L	VIII	?	5	none	+++	Greek
11	Tc.	W273L/W273L	VIII	?	?	none	+++	Greek
12	L.J.	W273L/W273L	VIII	10	7	none	+++	Bulgarian
13	H.A.	D198Y/W273L	VI/VIII			none	+++	Venezuelan
14	H.C	P397A/W273L	XII/VIII			none	+++	German
15	81RD354	IVS1+1G-T/W273L	I/VIII	7	?	none	+++	Finnish
16	81RD47	280G-T/W273L	II/VIII	8	5	none	+++	Dutch
17	Pe	Q408P/T500A	XII/XV	30	?	none	+++	French
18	C.J.	R148C/T500A	III/XV			+?	+++	English

The ß-Galactosidase-(GLB1) Gene

The structural **gene** for ß-Galactosidase is located on chromosome 3
More than **140 mutations** known, most of the patients have two different mutations **(„compound heterozygotes“)**



The Lysosomal Multienzyme Complex



The ß-Galactosidase-(GLB1) Gene

analyzed in more than 70 GM1 and MBD cases from Europe, Australia and South America

- Transient expression of mutations in cultured cells can be used to look whether enzyme can be made and if it is active or not.
- Fibroblast lines with the **same mutation** on both chromosomes („homozygotes“) can be used to study the function of mutated enzymes and their interaction with cellular metabolism

GM1
Y333H/Y333H
G190D/G190D
Y270D/Y270D
G438E/G438E
P397A/Q184R

Transient expression of mutations in COS-1 cells
Formation of stable gene products?
Enzyme activity?
Processing, conformation and transport of mutant proteins?

MBD
W273L/W273L
W273L/D198Y
W273L/P397A
Q408P/T500A

Transient Expression of GLB1-Mutants

abundance and activity of ß-gal mutants (Hofer et al. (2009) Hum Mut 30 (2009), 1214-21)

- Western blots show that all mutations result in the formation of normal amounts of enzyme protein

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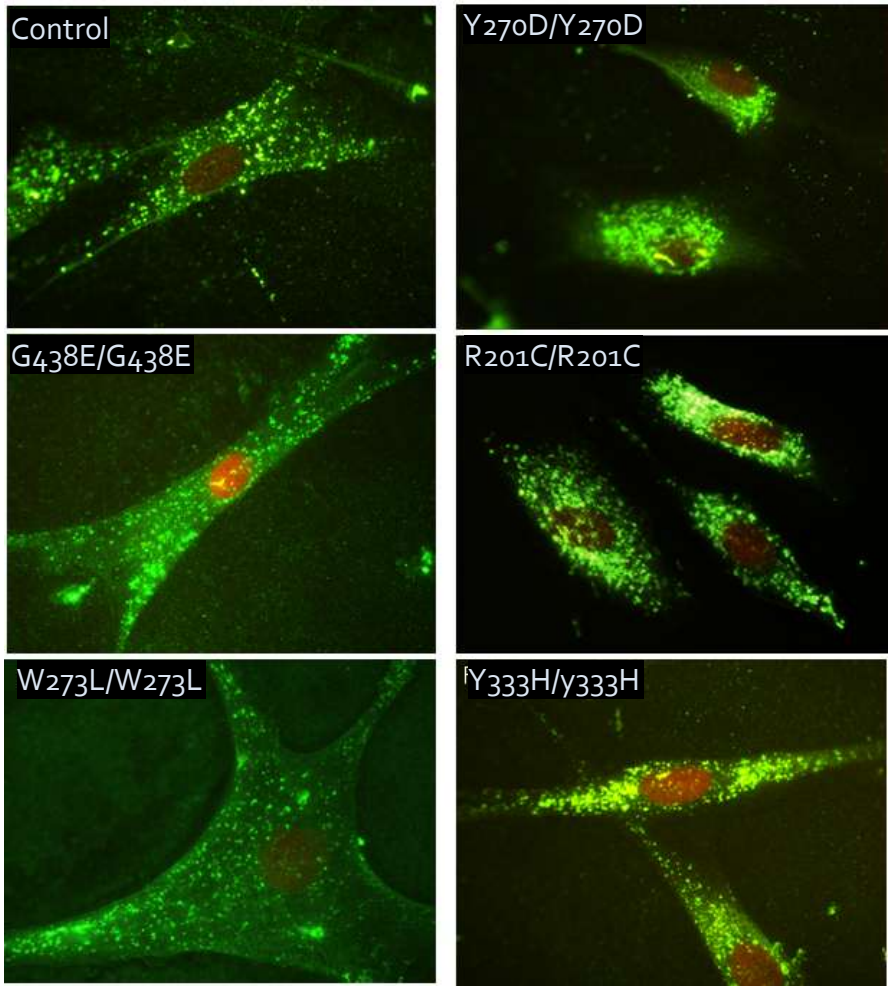
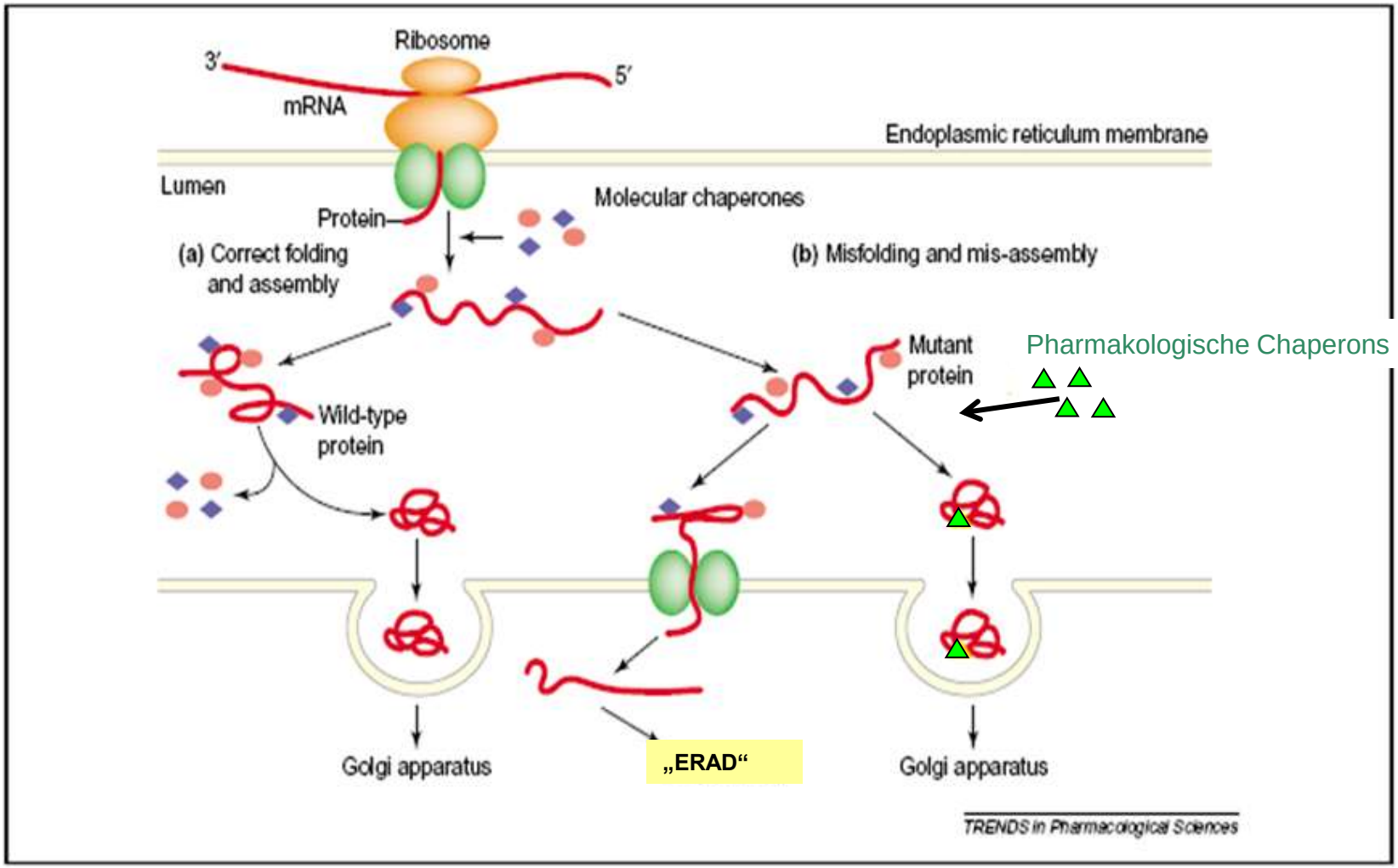
Homozygous GM1 and MBD fibroblasts

Immunofluorescence with monospecific antibodies



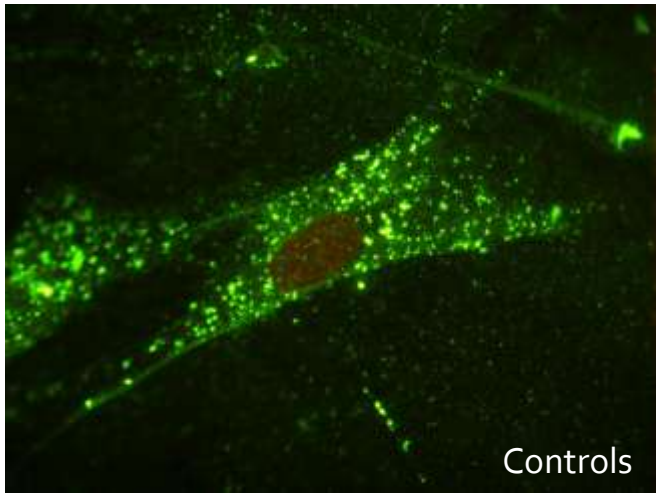
The Principle of Chaperon Therapy

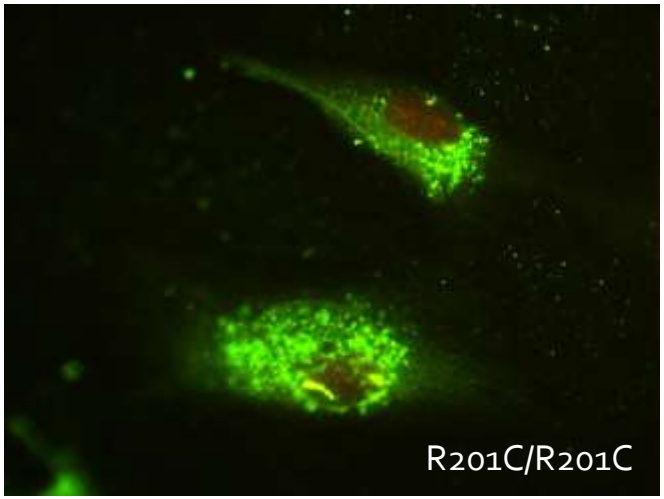
protein synthesis, folding and degradation



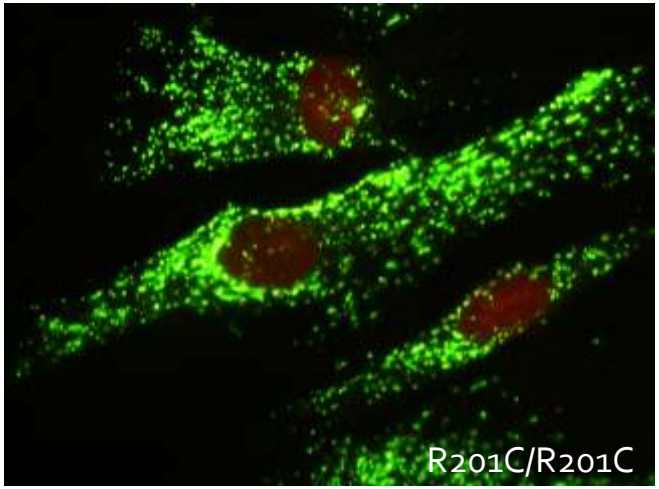
Pharmacological Chaperones

can repair the subcellular localisation of certain ß-gal mutants (Fantur et al 2009)





cultivated in the presence of DLHexDGJ for 4 days



Pharmacological Chaperones

can repair the activity of certain ß-gal mutants

Phenotype	Genotype	ß-gal increase (-fold)	% of controls
normal	WT	0,7-1,4	
infantile GM1	Y333H/Y333H	0,9-2,4	≤ 1,4 %
	P549L/P549L	0,8-1,7	≤ 0,9 %
	Y270D/Y270D	0,5-1,4	≤ 0,4 %
	A301V/A301V	0,8-1,3	≤ 1,9 %
juvenile GM1	R201C/R201C	6,2-18,5	≤ 74,7 %
	C230R/C230R	5,9-7,0	≤ 9,1 %
	R201H/R457X	9,2-15,1	≤ 29,7 %
	R201H/H281Y	~12	38 %
adult GM1	G438E/G438E	1,4-2,8	≤ 16%
Morquio B	W273L/W273L	0,7-1,5	≤ 5,6 %
Morquio B/ adult GM1	R201H/S149F	~15	36,8 %

Specific alleles for „skeletal“ phenotypes

summarized in Hofer et al., Clin Genet 2010

- **Sensitivity for chaperone treatment will be mutant-specific**
  - How can we predict the clinical phenotype from mutations?

- **Specific alleles for MBD**
  - Trp273 (W373L/W273L and W273L-heterozygotes, >18 (own) cases; W273R/H281Y);
  - Tyr500 (L173P/pT500A;N484K/T500A) ; 2 cases published
  - Tyr83 (pY83C/pD441N; Y83H/pR482C); 2 cases published

- **Specific alleles for adult GM1**
  - Tyr82 ; Five heteroallelic patients with delay of speech development, clumsiness with 2-4 years, spondyloepiphyseal dysplasia, ß-Gal approx. 3% of normal)

Examples for unclear alleles

clinical description of cases often contradicting

- Arg201
  - In homozygous patient **R210H/R201H** phenotype is **MBD** (?)
    (Santamaria et al., 2006, mainly genetic data)
  - In most heterozygote R201H phenotype is GM1 or „uncertain“
    (Hofer et al; 2009:R201H/S149F), MBD (Santamaria, 2006)

- Gly438
  - **G438E/G438E ;**
    one case GM1 (Roze et al;2005 full clinical report);
  - one case MBD (Bagshaw et al; 2002; mainly biochemical data)

Summary

Most of the known MBD patients carry two or at least one copy of a common allele, ***p.W273L*** *within their genome. So far,* ***no GM1 patient*** *with a W273L allele has been found. Most of GM1 patients are heterozygotes.*

Understanding of the effects of mutations on gene products and phenotypes is essential for a beneficial chaperone therapy. The W273L-derived enzyme protein is poperly transported and processed, but functionally impaired. Therefore chaperones are ineffectiv. While ***homozygotes for W273L*** *could* ***not*** *be treated a* ***chaperone-sensitive allele on the second chromosome would be promising*** *, e.g. one that impairs transport of precursors into the lysosomes.*

However, the current clinical classification is obsolete as the available data has been episodically collected since 30 years without systematic reference on genotypes, the natural history, rate of progression, range of disease severity, and distribution of specific symptoms in untreated patients.

This currently handicaps a clear concept on genotype-phenotype correlations despite considerable biochemical and genetic knowledge.

Furthermore, the identification of clinical endpoints or comparative data is needed for properly designed clinical trials

Open Questions

Clinical science

- *What is the natural history of MBD and late onset GM1 gangliosidosis ?*
- *What are the mutations on the GLB1 gene that cause Morquio phenotype and late onset GM1 gangliosidosis?*
- *What is the clinical spectrum of these mutations?*
- *What is the frequency of MBD and late onset GM1 gangliosidosis?*
- *Develop a novel classification system considering clinical **and** biochemical knowledge*

Basic science

- *What are the effects of mutations on enzyme function, subcellular distribution, multienzyme complex assembly?*
- *Molecular modelling of ß-gal and the multienzyme complex*
- *Pathogenesis of GM1 and MBD*
- *Chaperone sensitive mutations*
- *Handling of chaperones within the cells*

What is Morquio B?

The first MBD patient has been described in 1976. Only few have been reported thereafter.
Have the diagnostic criteria always been the same?

What is Morquio B:
Morquio-like Dysostosis multiplex (obligatory **without** CNS involvement)

or

late onset GM1 gangliosidosis with skeletal involvement?

Genotype-phenotype relations in inherited deficiency of ß-galactosidase (GM1-gangliosidosis, MPS IVB)

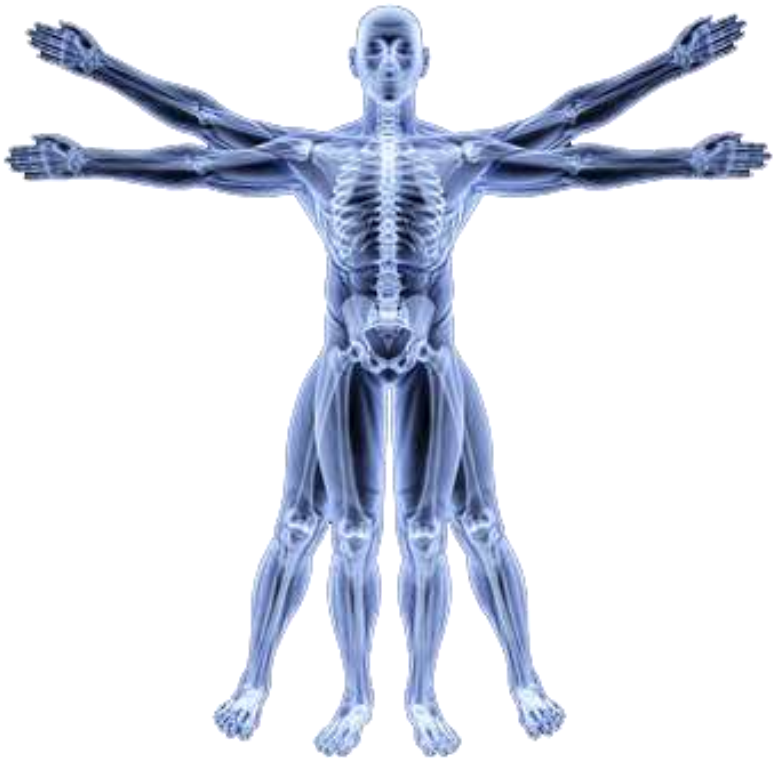
Name, Initials	
Date of birth	
Ethnic origin	
consanguinity	
Clinical phenotype infantile, juvenile, adult	
Age of onset month, years	
Presentation at onset most prominent symptoms	
Age at diagnosis	
Skeleton dorsolumbar kyphosis, gibbus, joint stiffness, dysostosis multiplex, spondylodysplasia, etc. ***Age*** at last exam	
Liver/spleen normal/enlarged	
Heart cardiomyopathy, thickening of valves	
Nervous system hypo/hypertonia, spasticity, ataxia extrapyramidal signs, psychomotor delay, intelligence	
Height, weight, head circumference ***Age*** at last exam	
Eye cherry red spot, corneal clouding	
MPS excretion total MPS, fractions (keratan sulfate detectable?)	
Oligosaccharides specific oligosaccharides?	
residual enzyme activity (% of normal)	
course: Short description, • living independently, ambulatory, present age etc. • age at loss of skills in GM1 • age at joint displacement in MPS IVB School performance, or profession, handicap in every day life, offsprings etc.	

The Morquio Better Project

Funded by the Canadian MPS Society

MBD and late onset GM1 gangliosidosis *are rare conditions with important impact on lysosomal pathophysiology .*

The project aims at establishing an international MBD patient registry and research databank.



Who is involved?

The Priest Family of Vancouver

Raised the entire funds for the project in support of their son who has Morquio B.

The Canadian Society for Mucopolysaccharide & Related Diseases Inc.

MPS

Canadian Society for Mucopolysaccharide and Related Diseases

Gesellschaft für MukoPolySaccharidosen und ähnliche Erkrankungen www.mps-austria.at

mps

Austrian Society for Mucopolysaccharidoses and Related Diseases

TIDE BC

BC CHILDREN'S HOSPITAL

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Medizinische Universität Graz

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Laboratory of Metabolic Diseases
Department of Pediatrics
Medical University of Graz Austria

Specific questions and aims

Aims

To identify the patients, physicians, and scientists that are caring for these patients, providing diagnosis and carrying out research on MBD and late infantile GM1 gangliosidosis.

The registry will involve two tiers

*A **patient database** containing demographic and core clinical data ,*

including data on caring physicians

*A **research database** containing detailed clinical biochemical, and molecular genetic data, providing access to staadadized information world-wide*

***Proposed work** shall include*

- *to assemble a **worldwide group of experts** to oversee the registry/databank*
- *to **design and implement the registry/databank***
- *to begin* collecting patient data *and **populating the registry/databank***
- *to design and implement a **public website for sharing information and educational materials** about MBD*

The websites

Priest family website

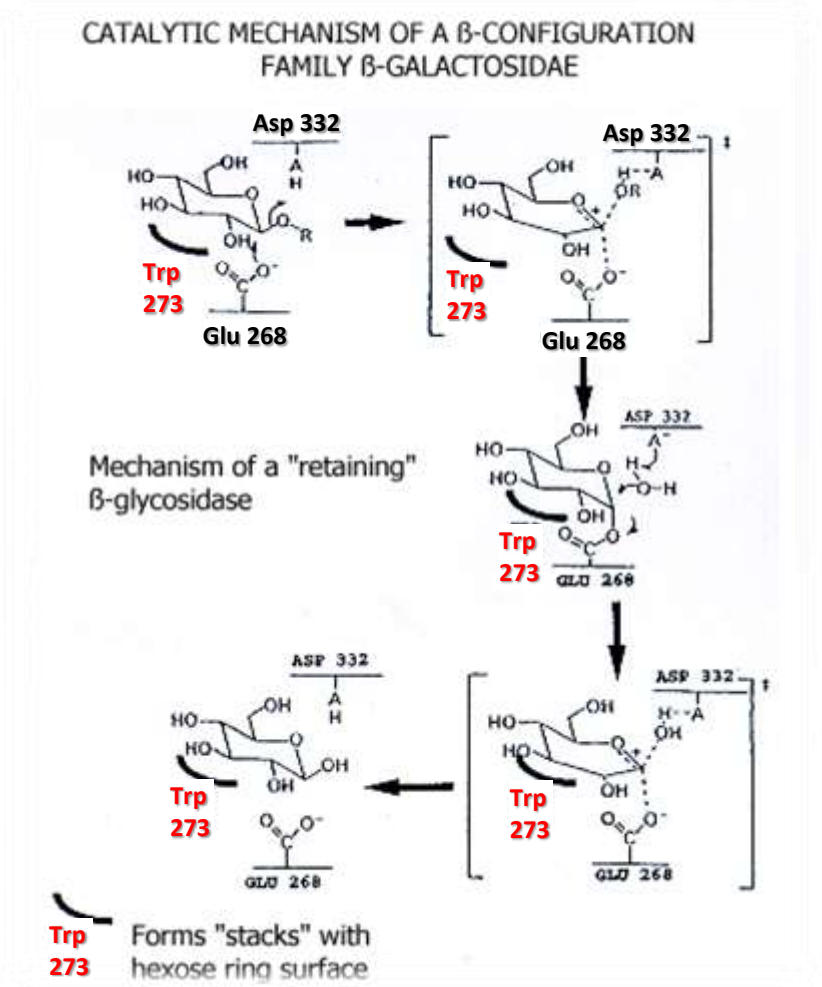
www.morquioB.com

Morquio B Registry

www.morquioB.org

Pathogenesis of Morquio B -

a „kinetic variant“?



Paschke et al. (2001):
A single two-base mutations Trp273Leu (W273L) is present in **14 of 15 patients** with Morquio B

Callahan et al. (2000):
The substrates of ß-gal are bound to two two acidic residues in exon 8, Asp332 and Glu268

Trp273 is proposed to be required for „carbohydrate positioning“

Residual activity and COS cells

Y333H and R201H

- Y333H/Y333H
  - Late infantile GM1
  - Located in exon 10, adjacent to catalytic Asp332
  - Lysosomally located in fibroblasts
  - 3% of WT in COS1 cells
  - 1% of WT in fibroblasts
  - Not chaperone sensitive

- R201H/R201H
  - R201H/R201H reported as MBD (?)
  - Located in exon 6, distant from catalytic site
  - No lysosomal localization of gene products
  - 36,2 % of WT in COS cells
  - >3% in fibroblasts
  - Chaperone sensitive

subcellular location *rather than* ***activity of gene product*** *determinant for phenotype?*

- Y333H/R201H
  - Juvenile GM1, **not MBD (!!)**
  - therefore the „more severe“ allele Y333H would determine phenotype
  - 1% of WT in fibroblasts