# Gene Expression-Targeted Isoflavone Therapy: Facts, Questions and Further Possibilities

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*University of Gdansk*

*Gdansk, Poland*

UNIWERSYTET GDAŃSKI

# Lysosomal storage diseases (LSD)

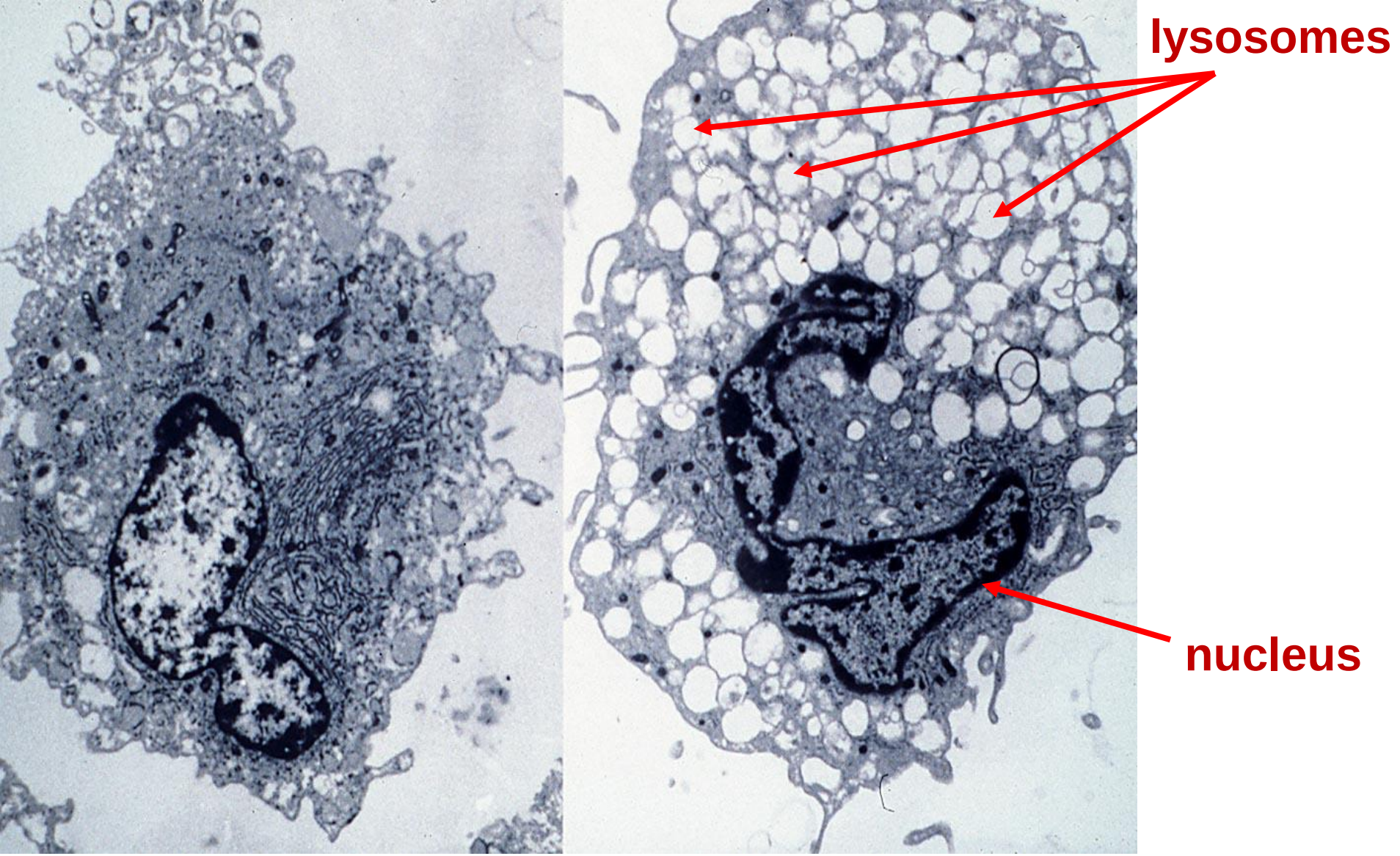
- A group of over 50 diseases
- Each disease is caused by a deficiency in:

(i) a specific lysosmal hydrolase (leading to an inability to degrade particular macromolecules, for example: sphingolipids, glycoproteins, glycosaminoglycans)

(ii) a protein involved in transport of particular compounds through lysosomal membranes

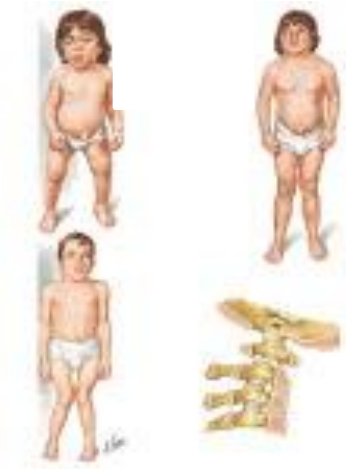
(iii) an enzyme that modifies lysosomal proteins, ensuring their proper localization and function

(iv) an activator of particular lysosomal enzyme




Normal fibroblast LSD fibroblast

# The deposition of undegraded substrates in tissues throughout the body leads to a multisystemic disease



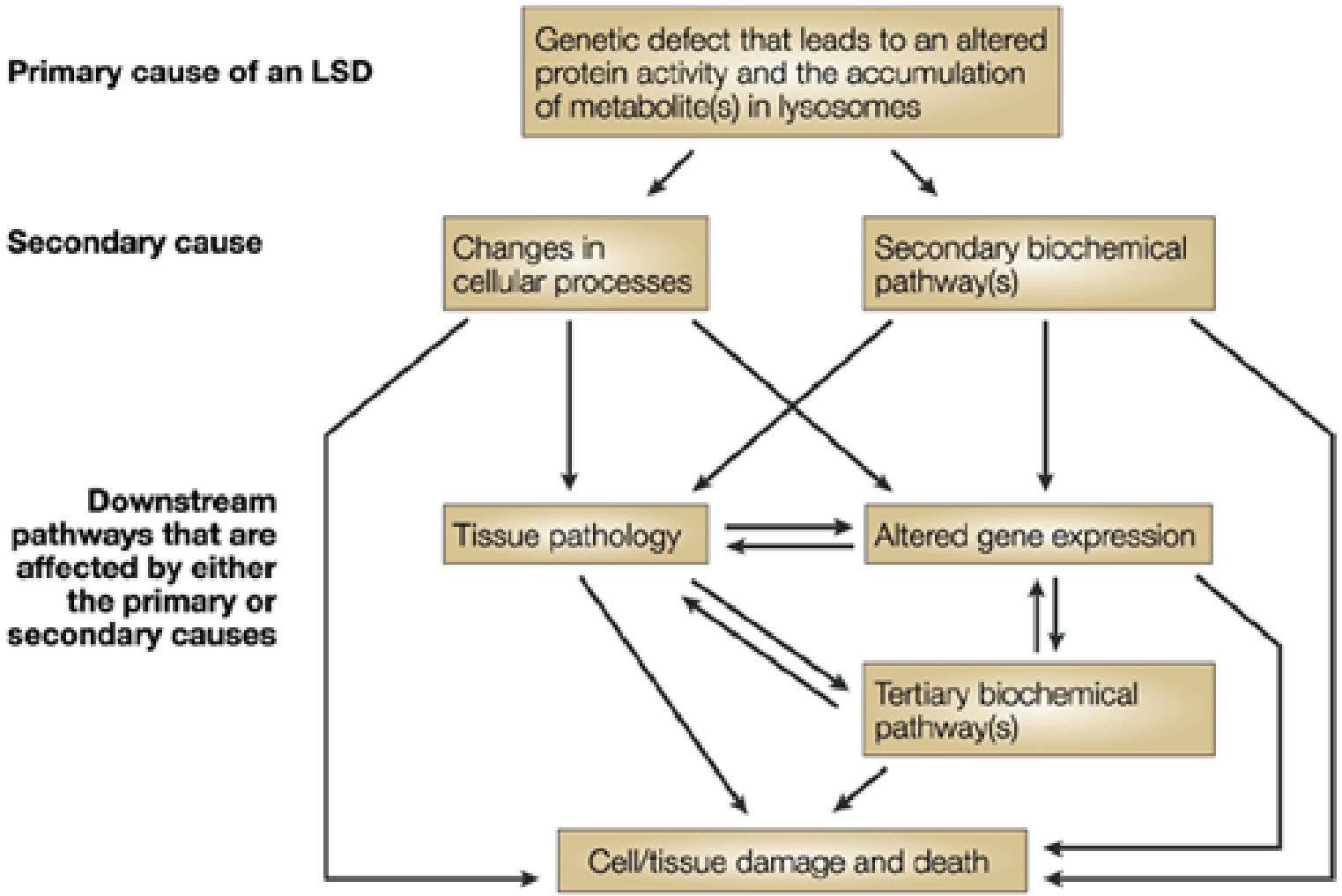
Growth deficiency
(dwarfism)

Central nervous system
and sensory organs affetced

Bones and joints problems
(*Disostosis multiplex)*

Visceral organs' dysfunctions

# Pathogenic cascades in LSD




Nature Reviews | Molecular Cell Biology

# Mucopolysaccharidoses (MPS): a group of lysosomal storage diseases

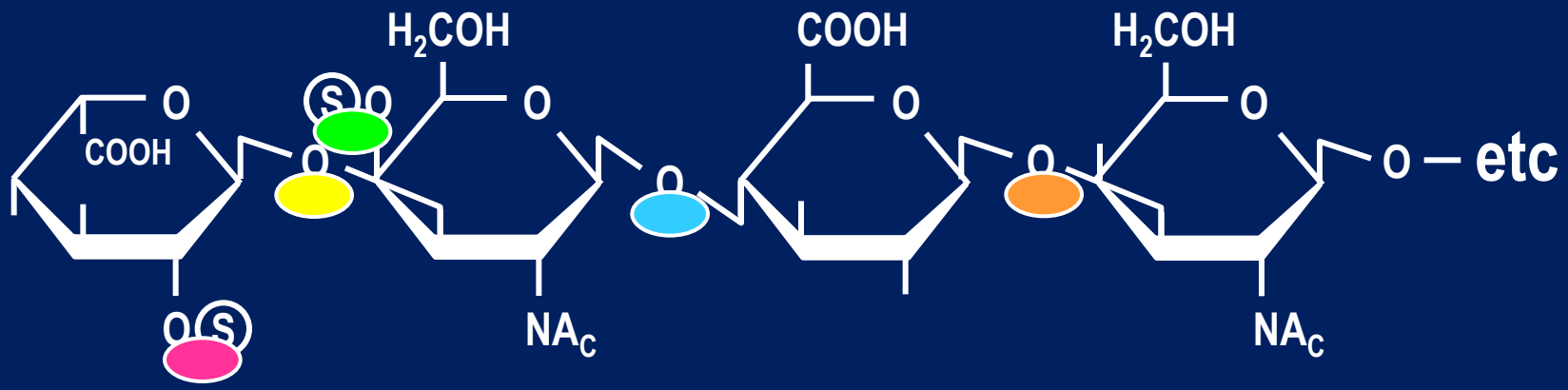
**Glycosaminoglycans (GAGs) are accumulated in MPS**

**Different kinds of GAGs, whose degradation is inhibited at different steps, are accumulated in various MPS types.**

# Degradation of dermatan sulfate

- I2S enzyme
- α-L-iduronidase
- N-acetylgalactosamine 4-sulfatase
- β-hexosaminidase A, B, S
- β-glucuronidase

I2S = iduronate-2-sulfatase
NAc = N-acetylgalactosamine



# MPS are chronic, progressive and life-threatening diseases

## Clinical features include:

- organomegaly (e.g. liver, spleen, tongue)
- dysostosis multiplex
- obstructive airway disease
- impaired cardiovascular functions
- impaired hearing and vision
- joint stiffness
- hernias
- spinal cord compression
- hydrocephalus
- mental retardation (in some cases)

SINGNIFICANY SHORTENED LIFE SPAN (death usually within a childhood)

# Potential therapies for LSD:

- Enzyme replacement therapy (ERT)

- Bone marrow (or stem cell) transplantation

- Gene therapy

- Stop codon read-through

- Small chaperones

- Substrate optimization therapy

- Substrate reduction therapy

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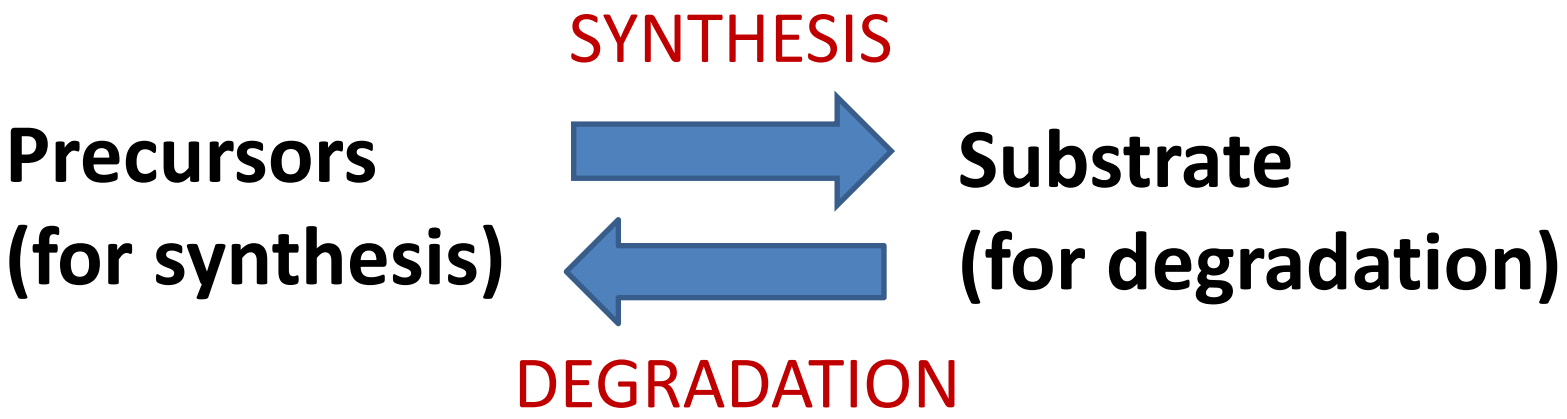
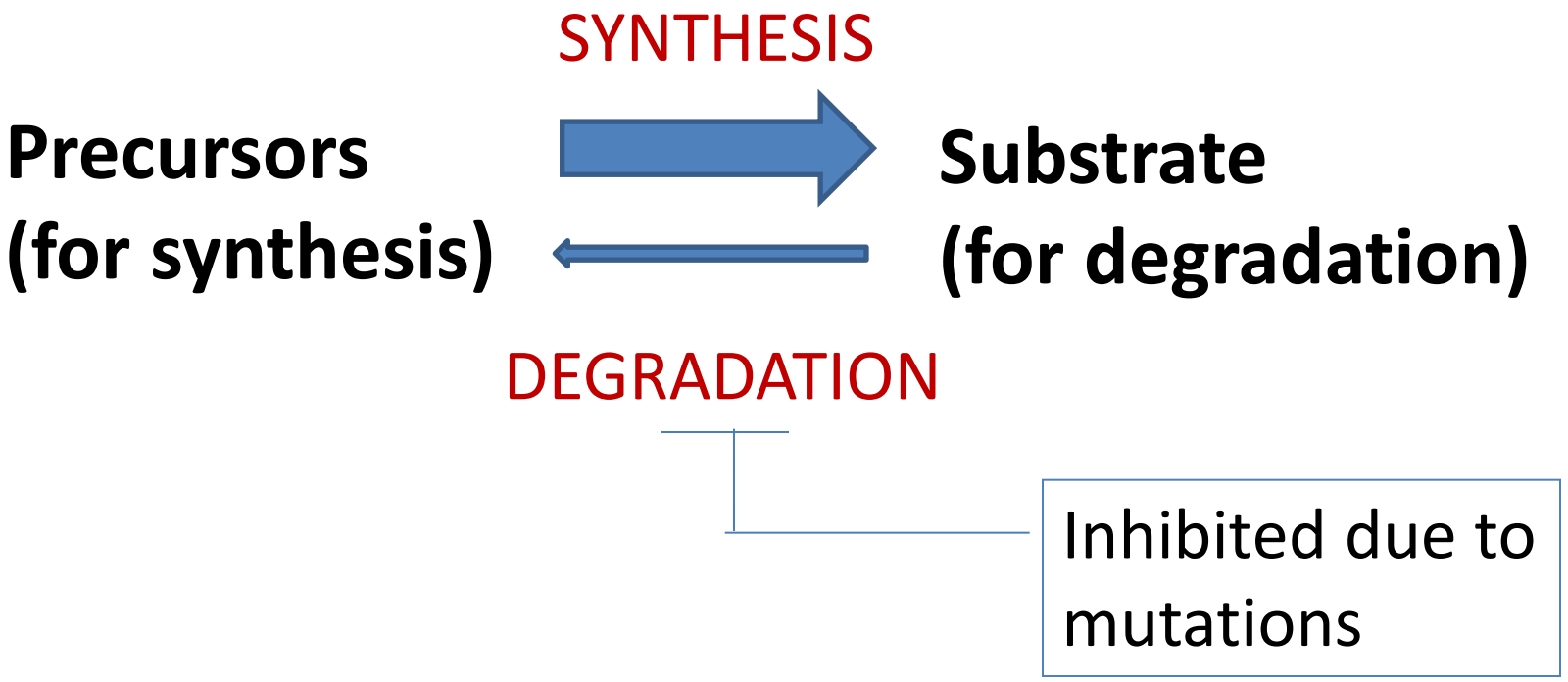
# The idea of substrate reduction therapy



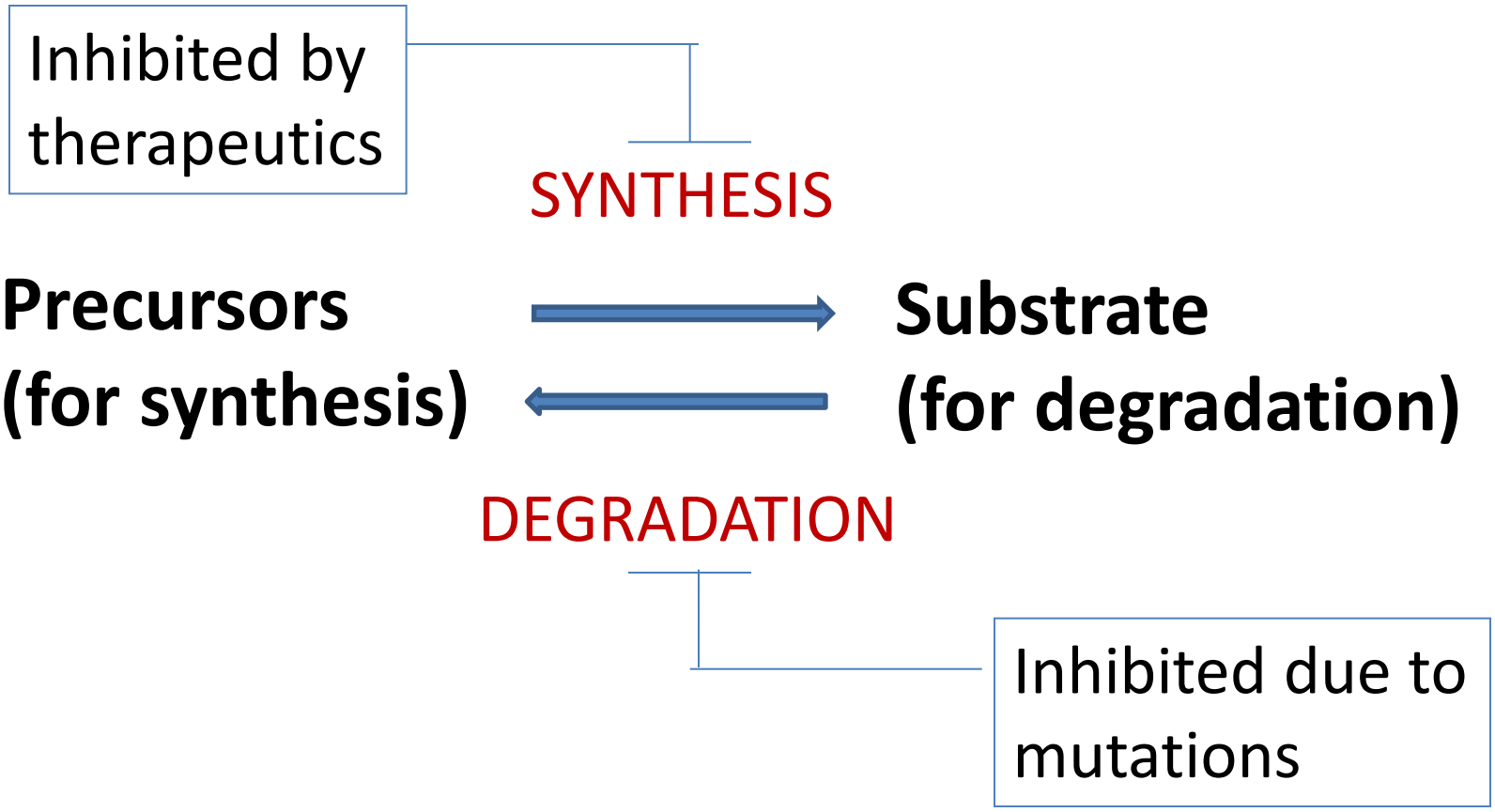

**NORMAL (Synthesis = Degradation)**

# The idea of substrate reduction therapy




**LSD (Synthesis > Degradation)**

# The idea of substrate reduction therapy




**SRT for LSD (Synthesis = Degradation)**

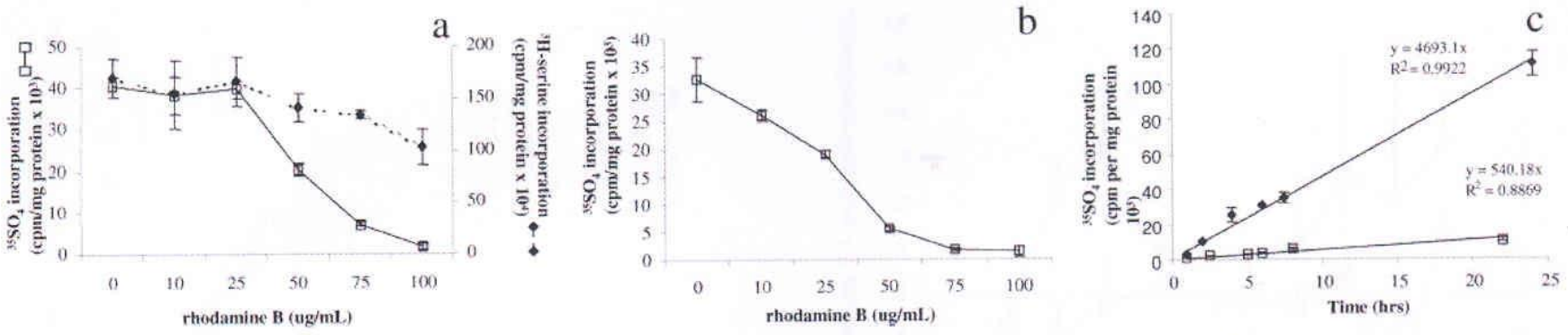
# Facts

PEDIATRIC RESEARCH

*Pediatric Research* (2006) **60,** 309-314

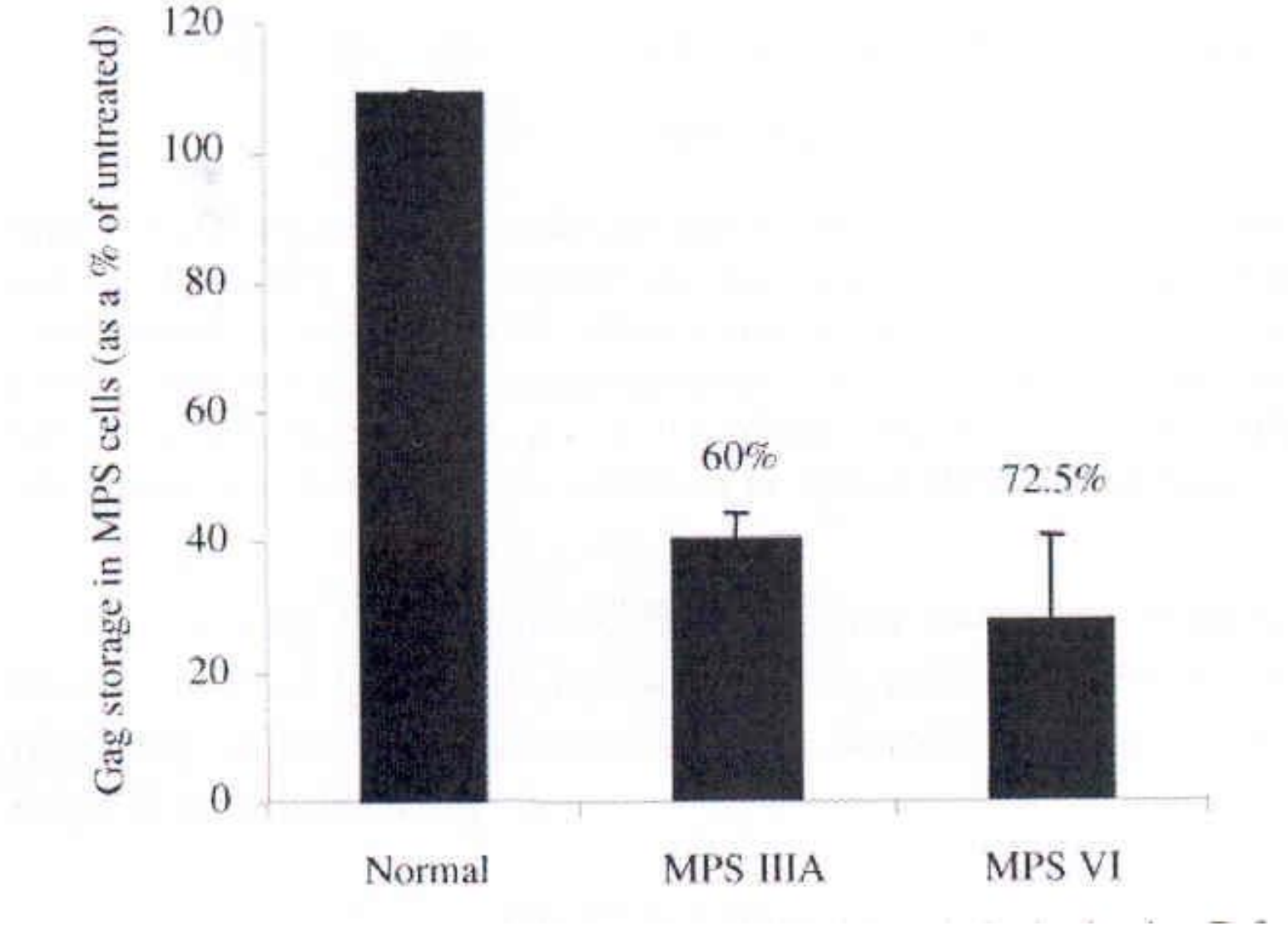
# Inhibition of glycosaminoglycan synthesis using rhodamine B in a mouse model of mucopolysaccharidosis type IIIA

Roberts A.L., Thomas B.J., Wilkinson A.S., Fletcher J.M., Byers S.




**Rodamine B is an inhibitor of GAG synthesis but the mechanism of its action in unknown**

# Reduction of GAG accumulation in MPS fibroblasts treated with rhodamine B for 14 days



**MPS IIIA mouse treated with rhodamine B (weekly infusions of 1 mg/kg) for 6 months**

**Untreated MPS IIIA mouse**

**Control (a healthy mouse)**

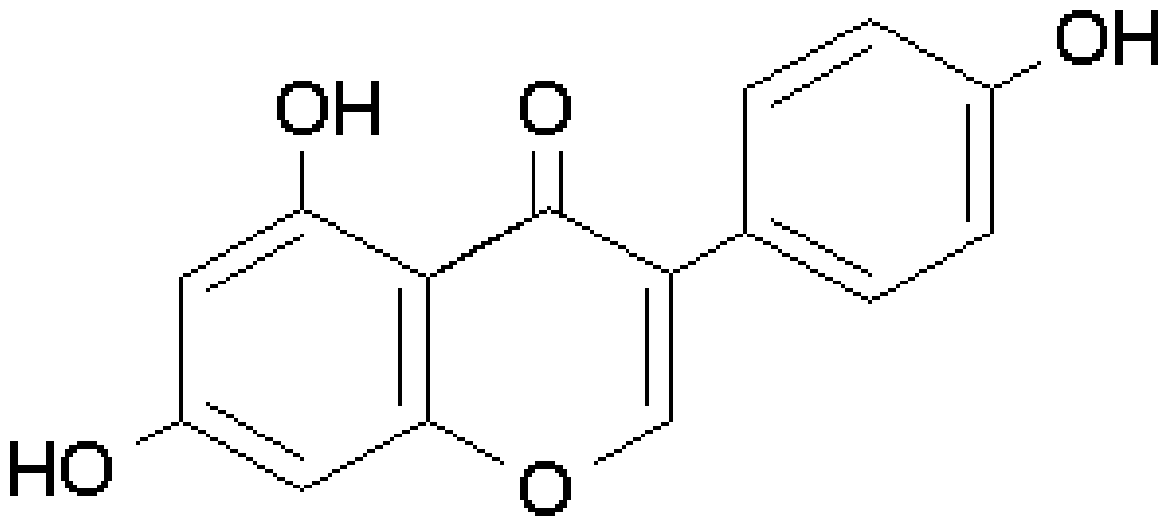
nature publishing group

European Journal of Human Genetics

*European Journal of Human Genetics* (2006) **14,** 846–852

# Genistein-mediated inhibition of glycosaminoglycan synthesis as a basis for gene expression-targeted isoflavone therapy for mucopolysaccharidoses

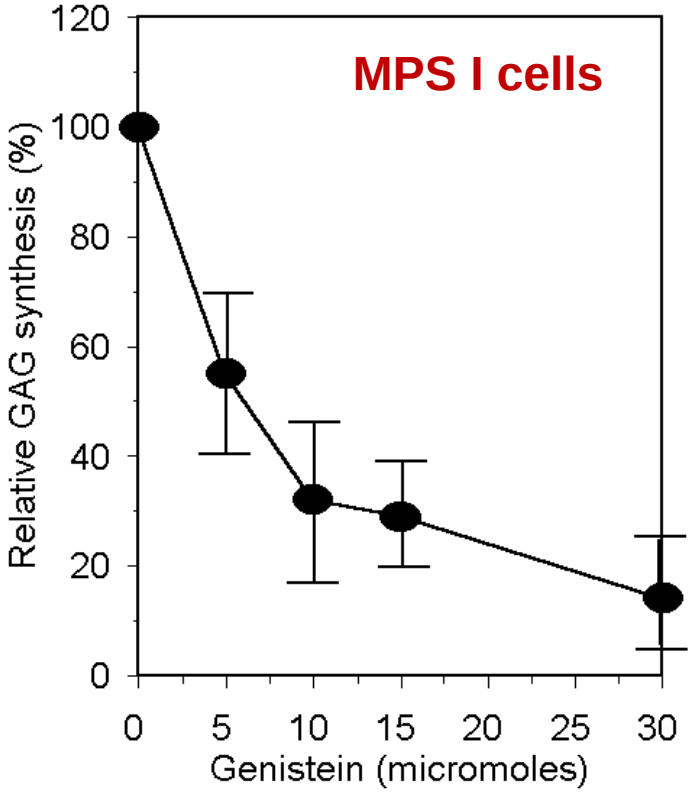
Ewa Piotrowska, Joanna Jakobkiewicz-Banecka, Sylwia Baranska,
Anna Tylki-Szymanska, Barbara Czartoryska, Alicja Wegrzyn and Grzegorz Wegrzyn



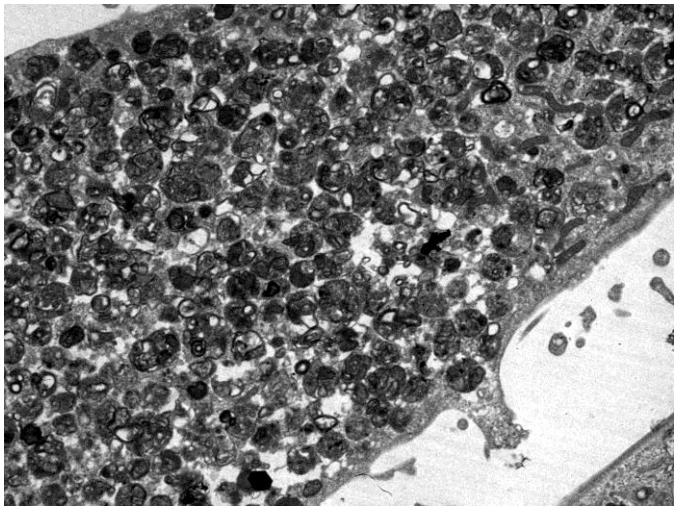

5, 7-dihydroxy-3- (4-hydroxyphenyl)-4*H*-1-benzopyran-4-one

4', 5, 7-trihydroxyisoflavone

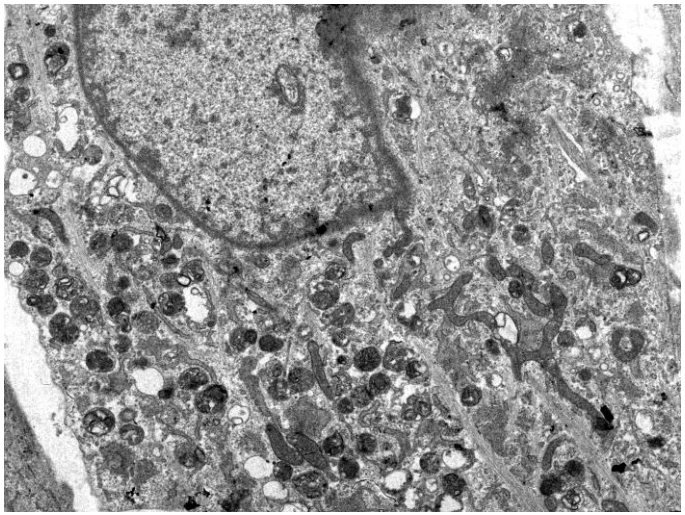
# Genistein




MPS IIIA cell



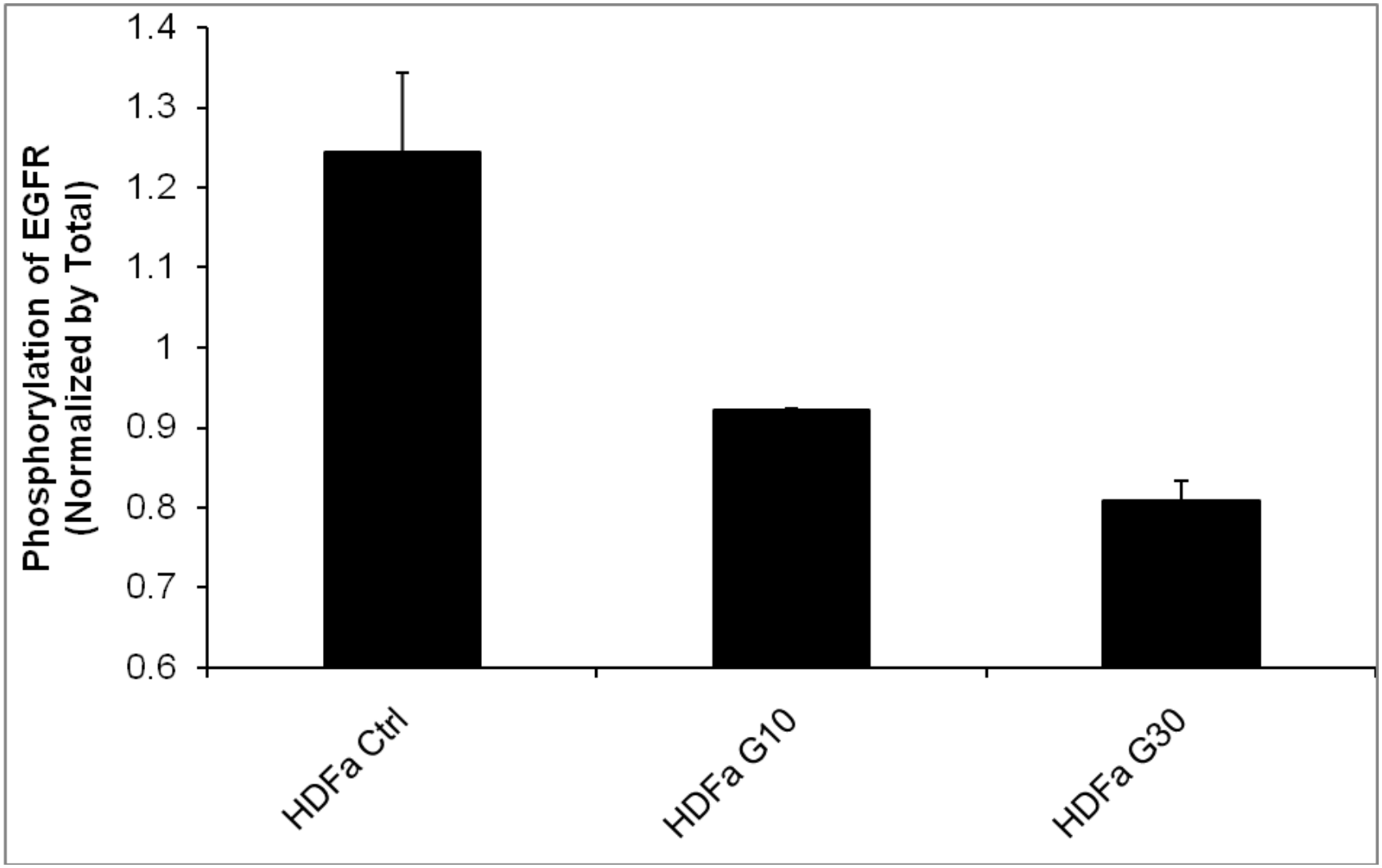
Genistein

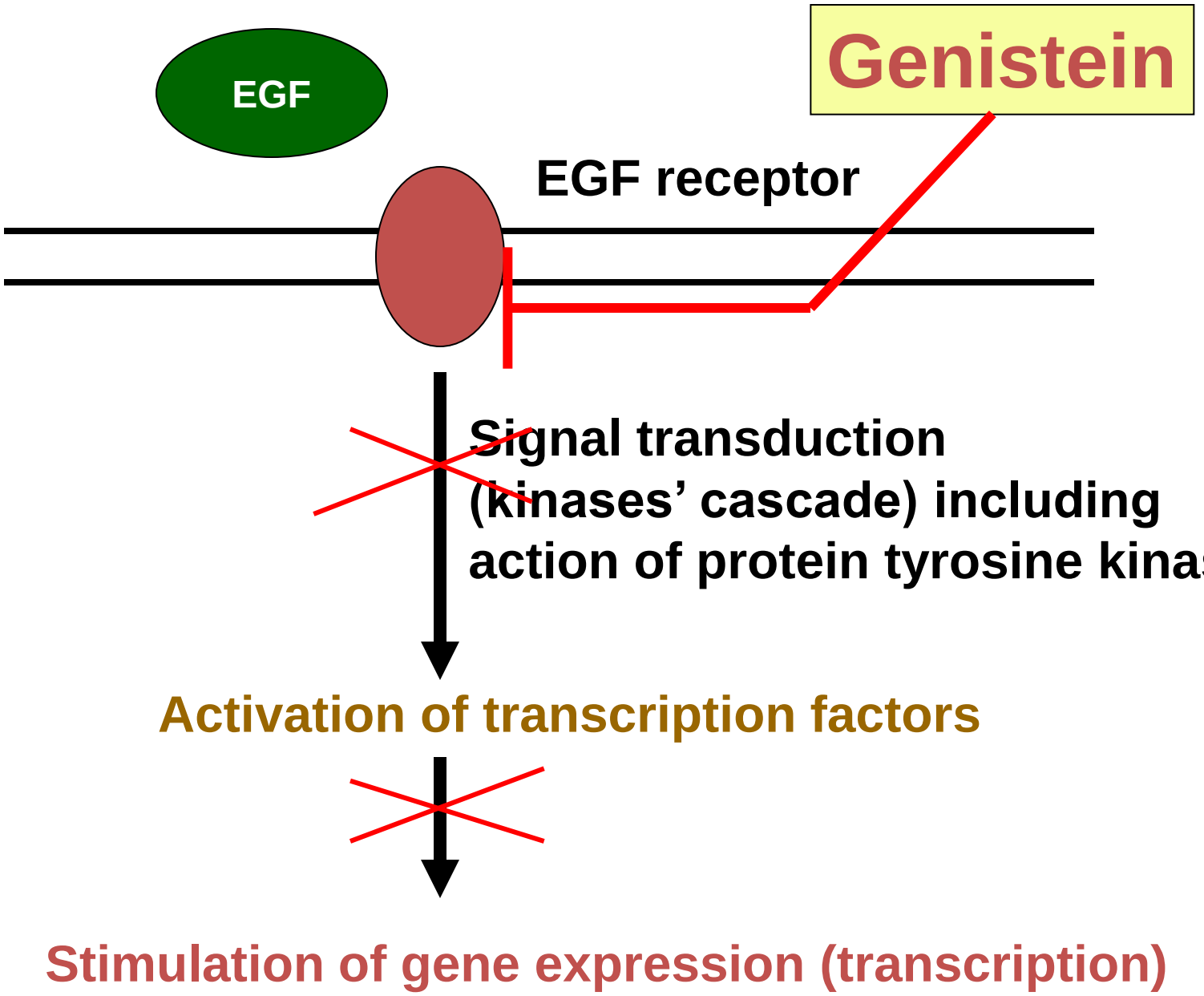


Inhibition of glycosaminoglycan (GAG) synthesis by genistein

Piotrowska et al. (2006) *Eur. J. Hum. Genet.* **14:** 846-852

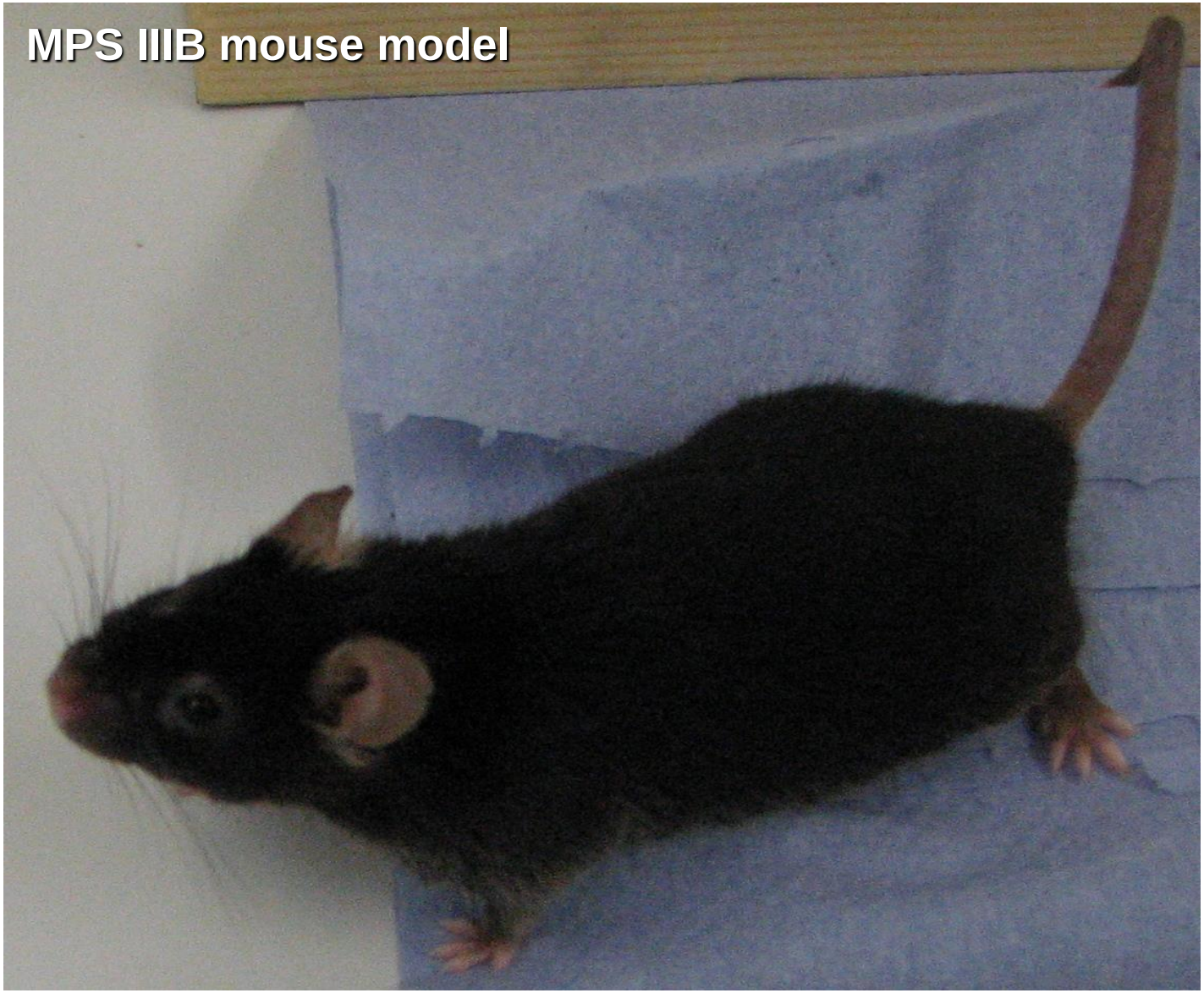
# Inhibition of EGF receptor phosphorylation (long term experiment - 24 h)





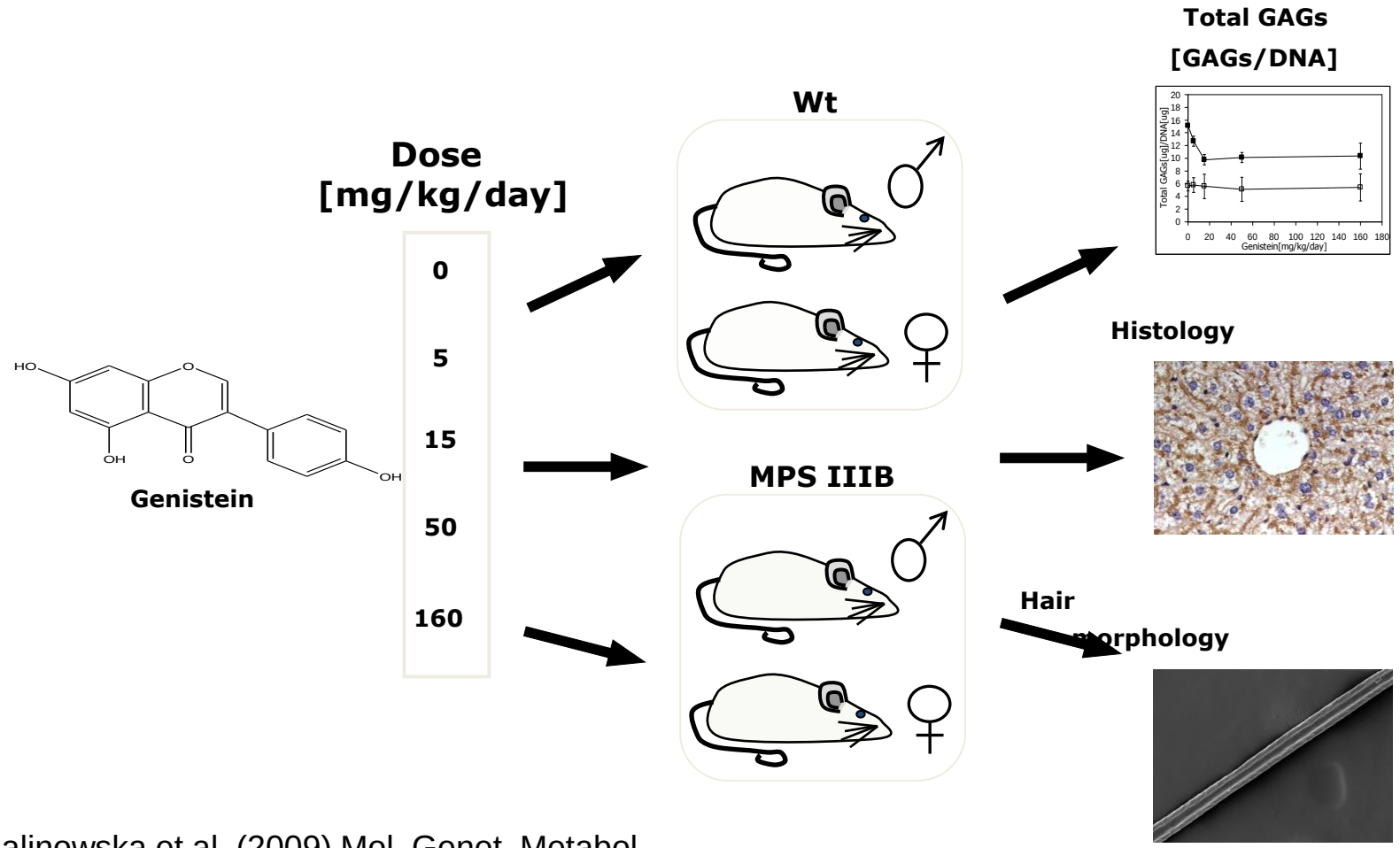

Jakobkiewicz-Banecka et al. (2009) *J. Biomed. Sci.* **16:** 26

# MPS IIIB mouse model



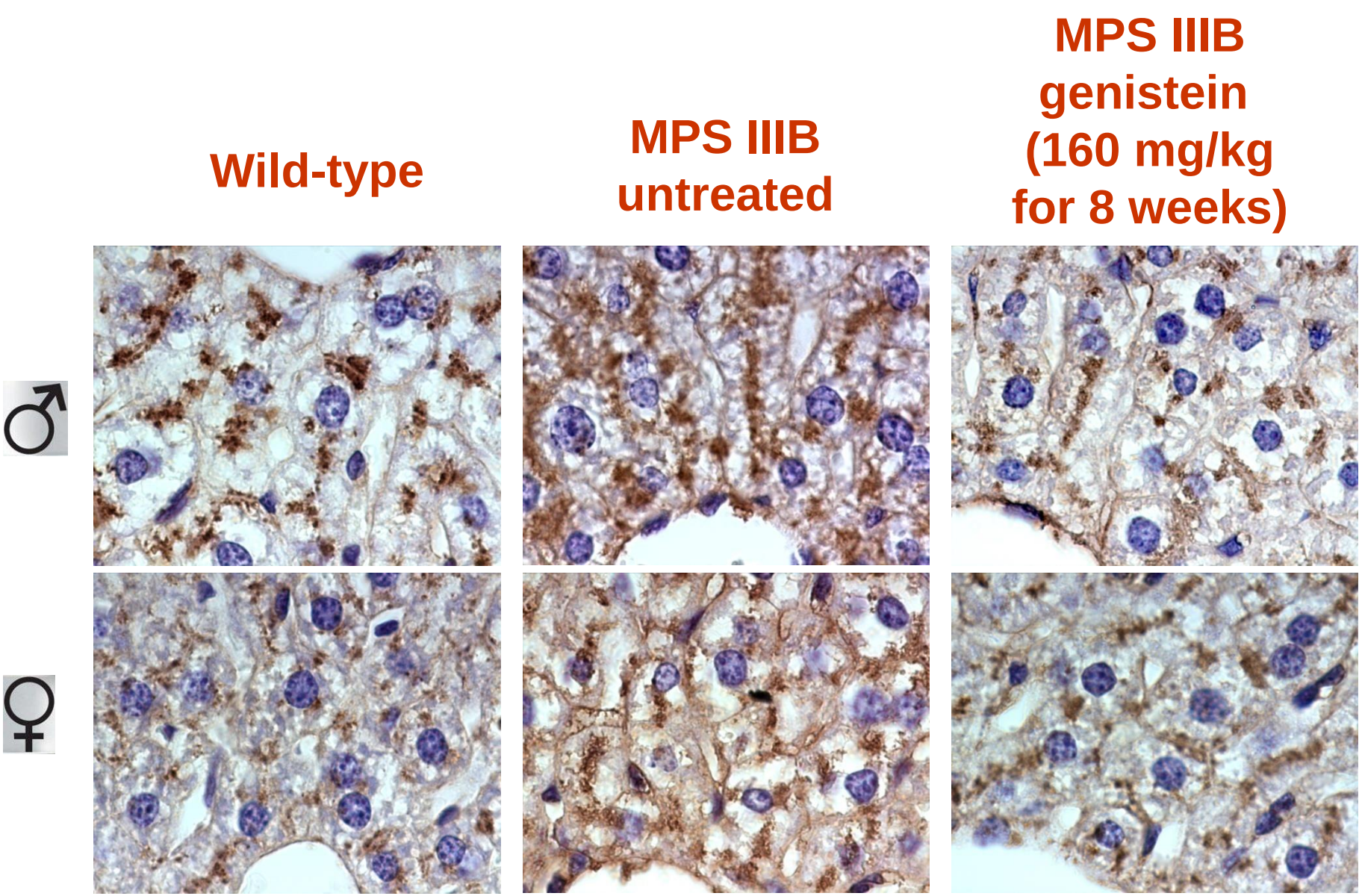
# Short-term experiment

**AIM: To** establish the most effective and non-toxic dose of genistein which can significantly reduce GAG storage in MPS IIIB mice and be used as substrate reduction therapy for Sanfilippo syndrome

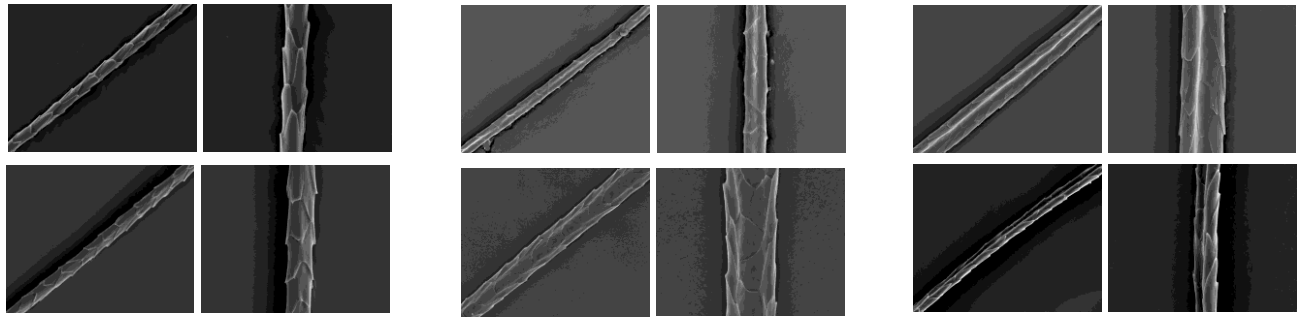


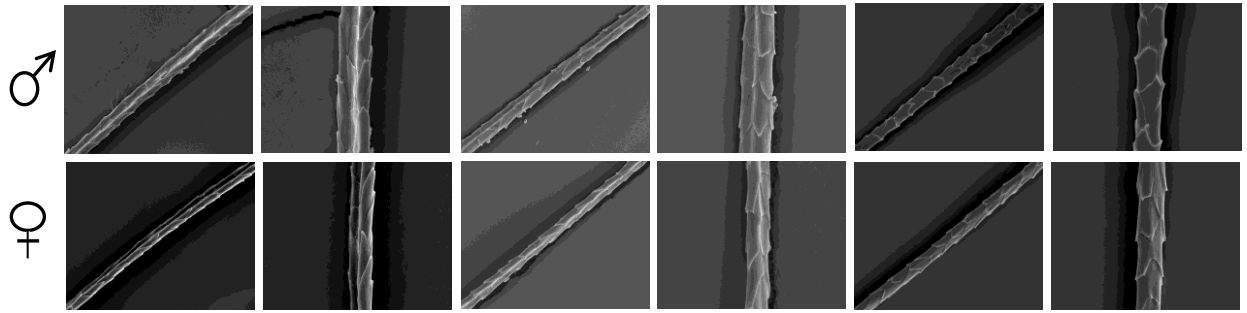

Malinowska et al. (2009) Mol. Genet. Metabol.

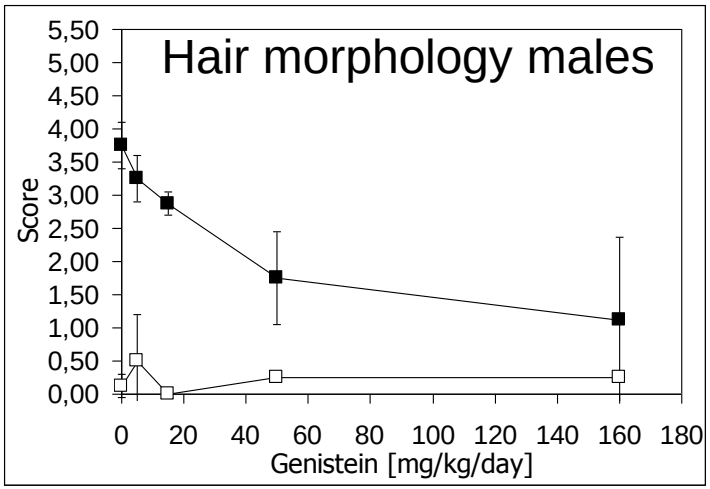
# Reduction of GAG storage in liver of MPS IIIB mice

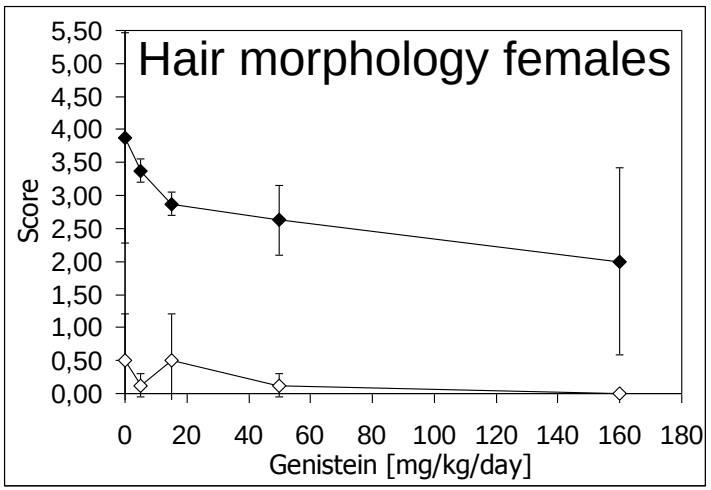


# Electron Microscopy

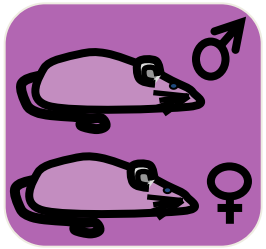


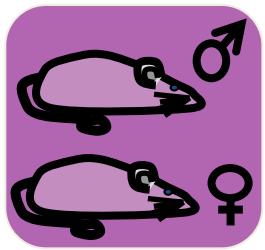


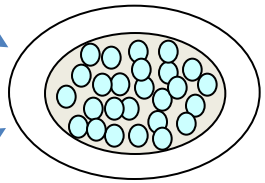



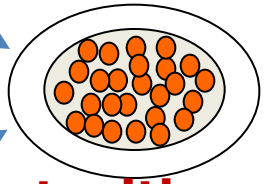
# Long-term experiment



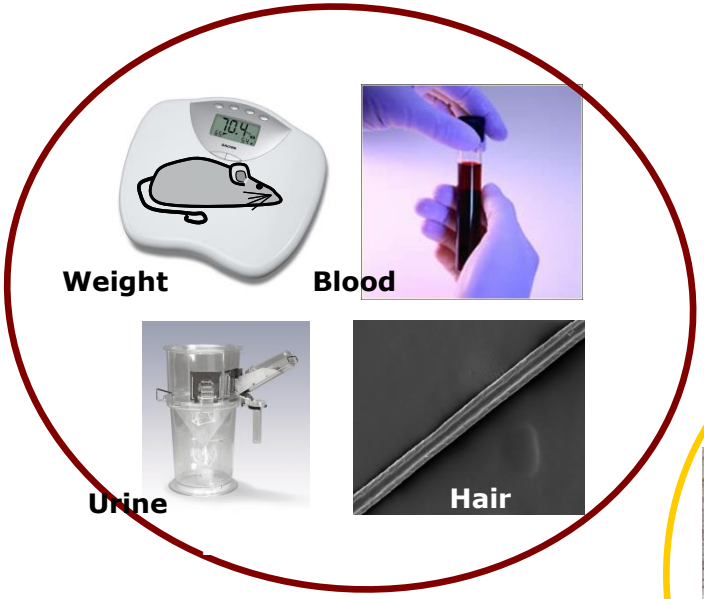


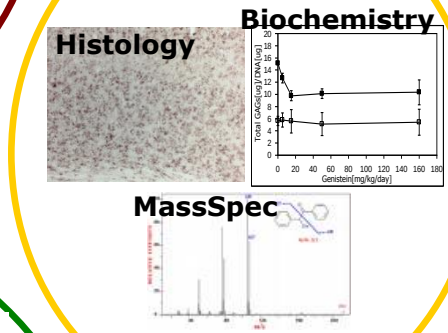


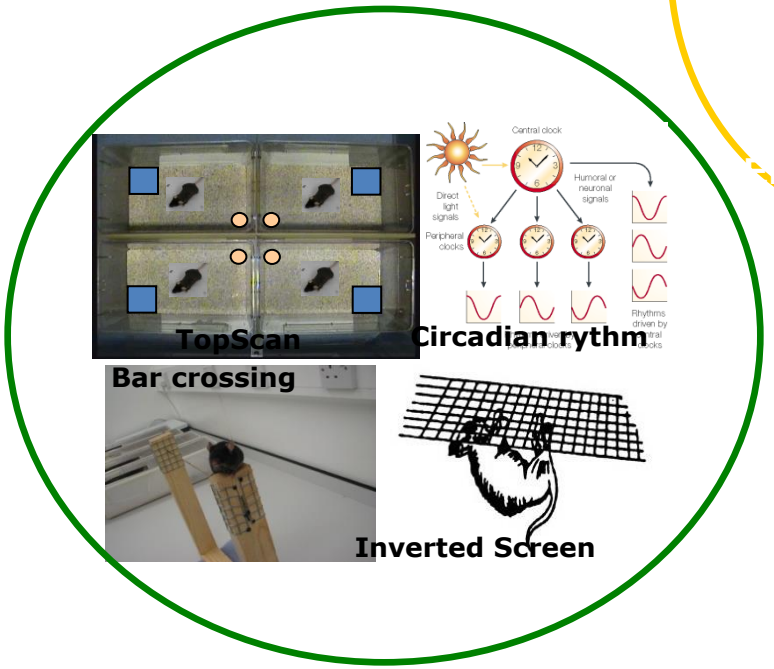
**Soy free diet**



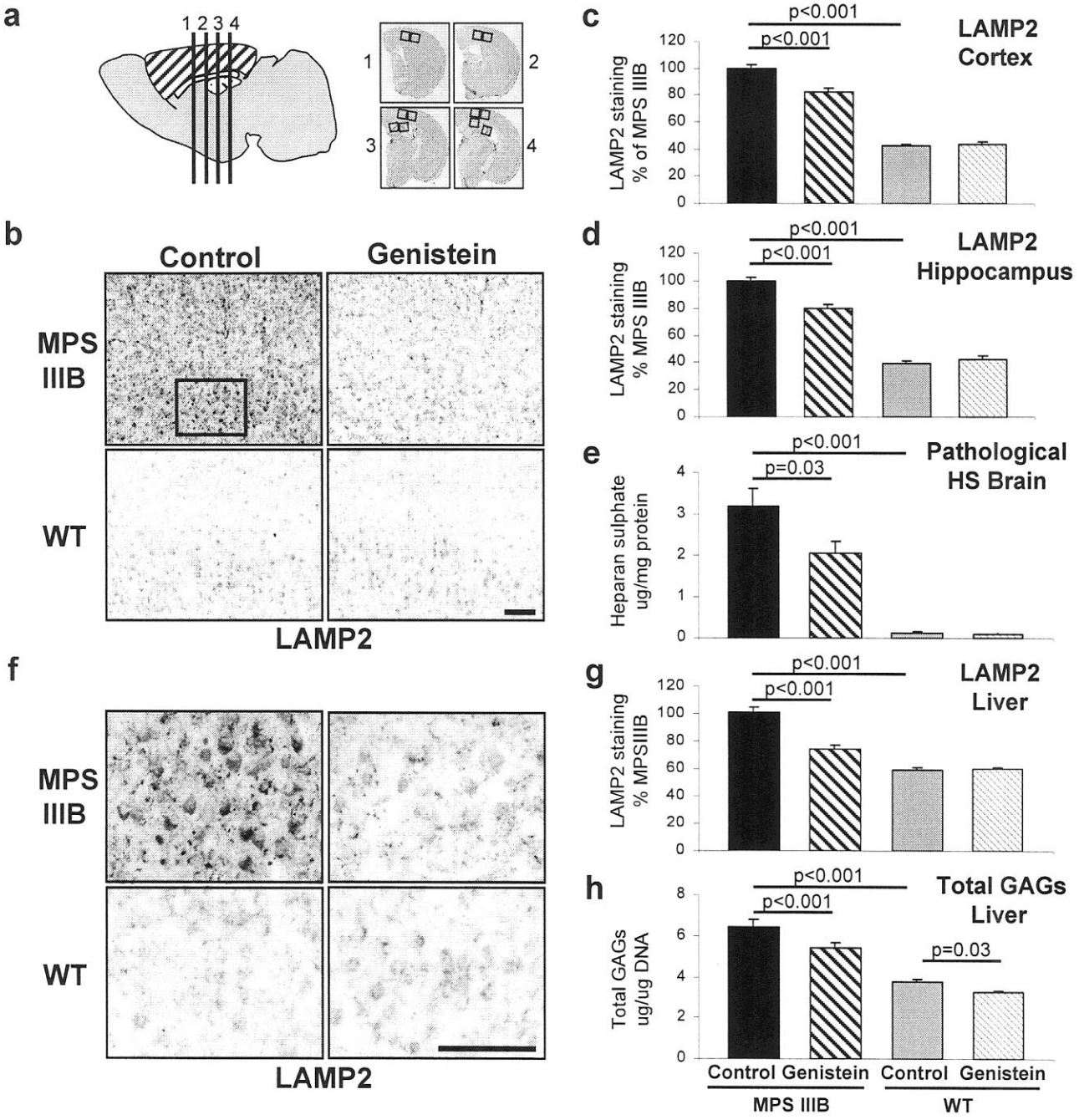
**Diet with genistein 160 mg/kg**

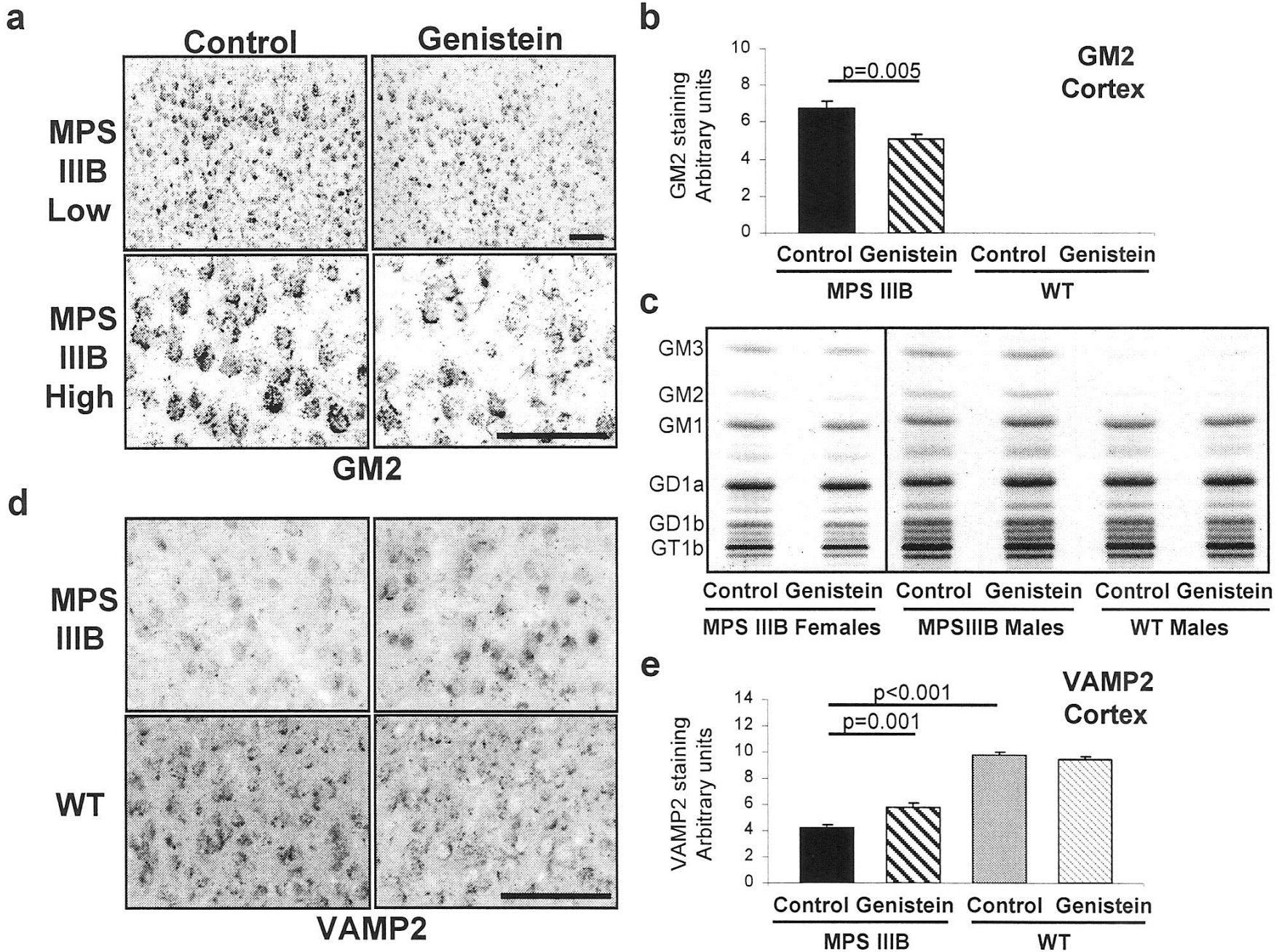


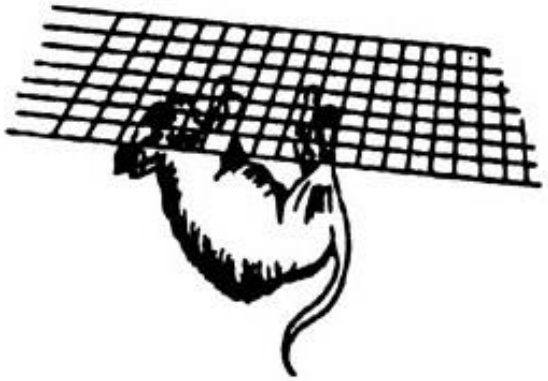




Malinowska et al. (2010) PLoS ONE

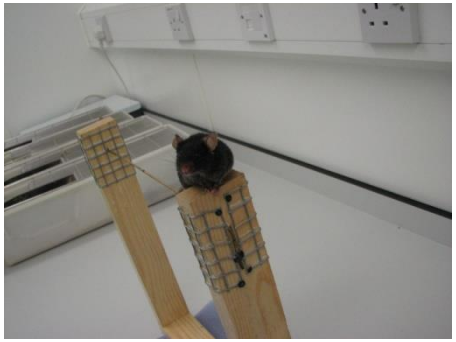

a
1 2 3 4
1
2
3
4
b
Control
Genistein
MPS IIIB
WT
LAMP2
c
p<0.001
p<0.001
LAMP2 staining % of MPS IIIB
LAMP2 Cortex
d
p<0.001
p<0.001
LAMP2 staining % MPS IIIB
LAMP2 Hippocampus
e
p<0.001
p=0.03
Heparan sulphate ug/mg protein
Pathological HS Brain
f
MPS IIIB
WT
LAMP2
g
p<0.001
p<0.001
LAMP2 staining % MPSIIIB
LAMP2 Liver
h
p<0.001
p<0.001
p=0.03
Total GAGs ug/ug DNA
Total GAGs Liver
Control Genistein Control Genistein
MPS IIIB
WT


a
Control
Genistein
MPS IIIB Low
MPS IIIB High
GM2
b
GM2 staining Arbitrary units
p=0.005
GM2 Cortex
Control Genistein Control Genistein
MPS IIIB
WT
c
GM3
GM2
GM1
GD1a
GD1b
GT1b
Control Genistein Control Genistein Control Genistein
MPS IIIB Females
MPSIIIB Males
WT Males
d
MPS IIIB
WT
VAMP2
e
VAMP2 staining Arbitrary units
p<0.001
p=0.001
VAMP2 Cortex
Control Genistein Control Genistein
MPS IIIB
WT

# Behavioral tests

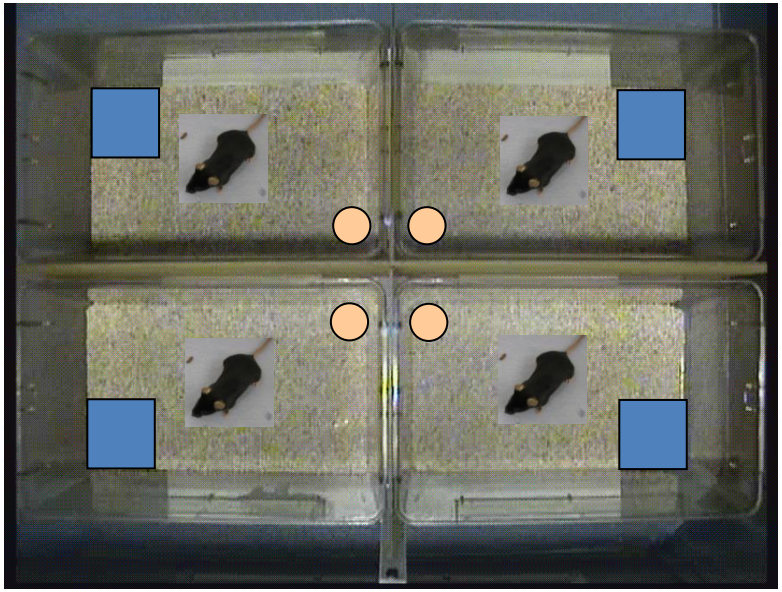


**Inverted screen test**

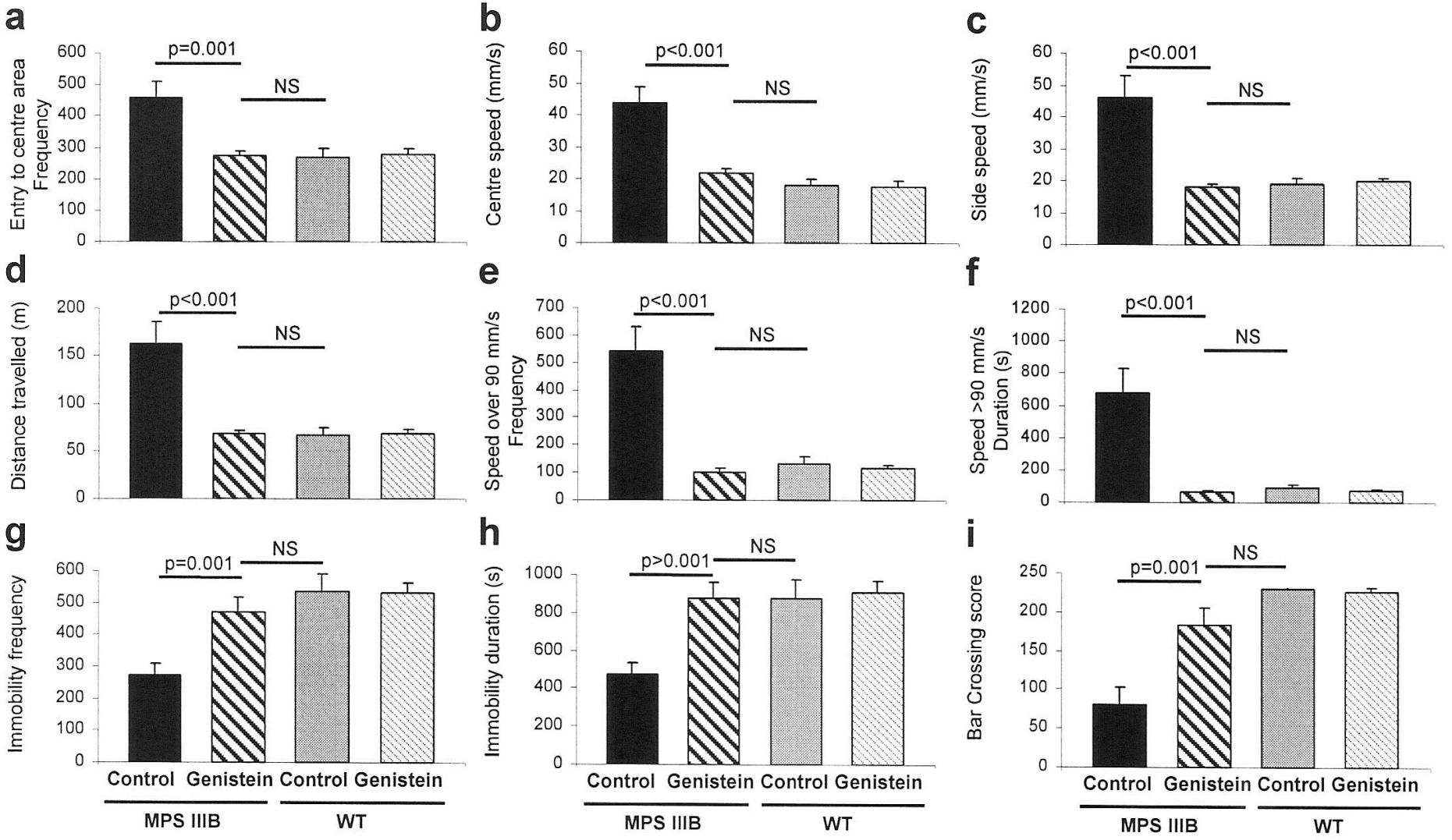


**Bar crossing test**

**Home cage behavior**



# Behavioral tests



THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 43, pp. 36283–36290, October 19, 2012
© 2012 by The American Society for Biochemistry and Molecular Biology, Inc. Published in the U.S.A.

# A Genetic Model of Substrate Reduction Therapy for Mucopolysaccharidosis*

Received for publication, July 21, 2012, and in revised form, August 24, 2012 Published, JBC Papers in Press, September 5, 2012, DOI 10.1074/jbc.M112.403360

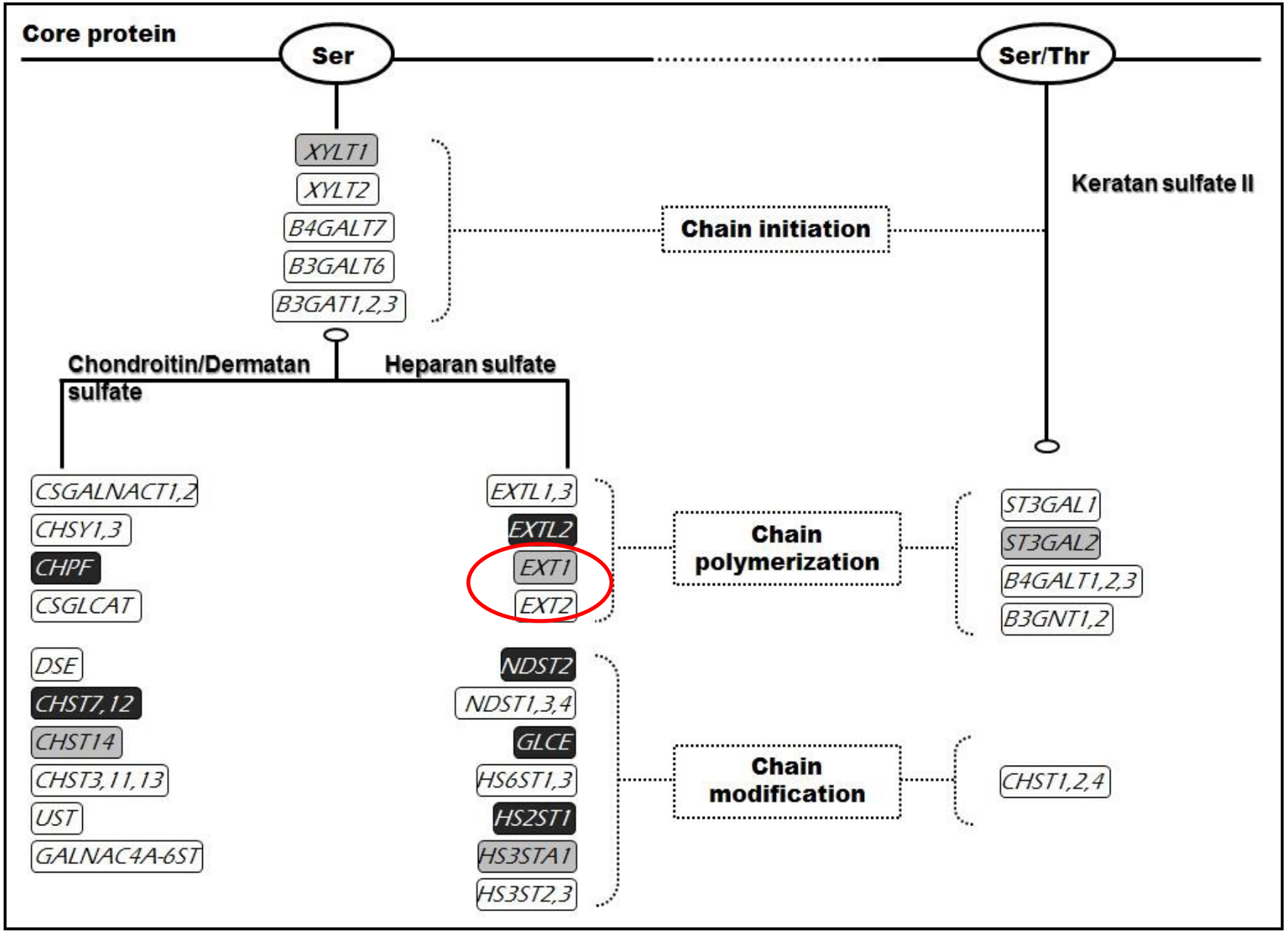
**William C. Lamanna**[‡], **Roger Lawrence**[‡], **Stéphane Sarrazin**[‡1], **Carlos Lameda-Diaz**[‡], **Philip L. S. M. Gordts**[‡], **Kelley W. Moremen**[§], **and Jeffrey D. Esko**[‡2]

*From the* [‡]*Department of Cellular and Molecular Medicine, Glycobiology Research and Training Center, University of California at San Diego, La Jolla, California 92093-0687 and the* [§]*Complex Carbohydrate Research Center, University of Georgia, Athens, Georgia 30602-4712*

**Background:** Treatment of neuropathology in mucopolysaccharidoses may be possible by substrate reduction therapy.
**Results:** A genetic model of substrate reduction therapy for mucopolysaccharidosis type IIIa ameliorates disease pathology in the brain.
**Conclusion:** Partial inhibition of glycosaminoglycan biosynthesis may be useful for treating mucopolysaccharidoses type III.
**Significance:** Proof of principle is presented that inhibition of heparan sulfate synthesis might prove beneficial for treating mucopolysaccharidoses.

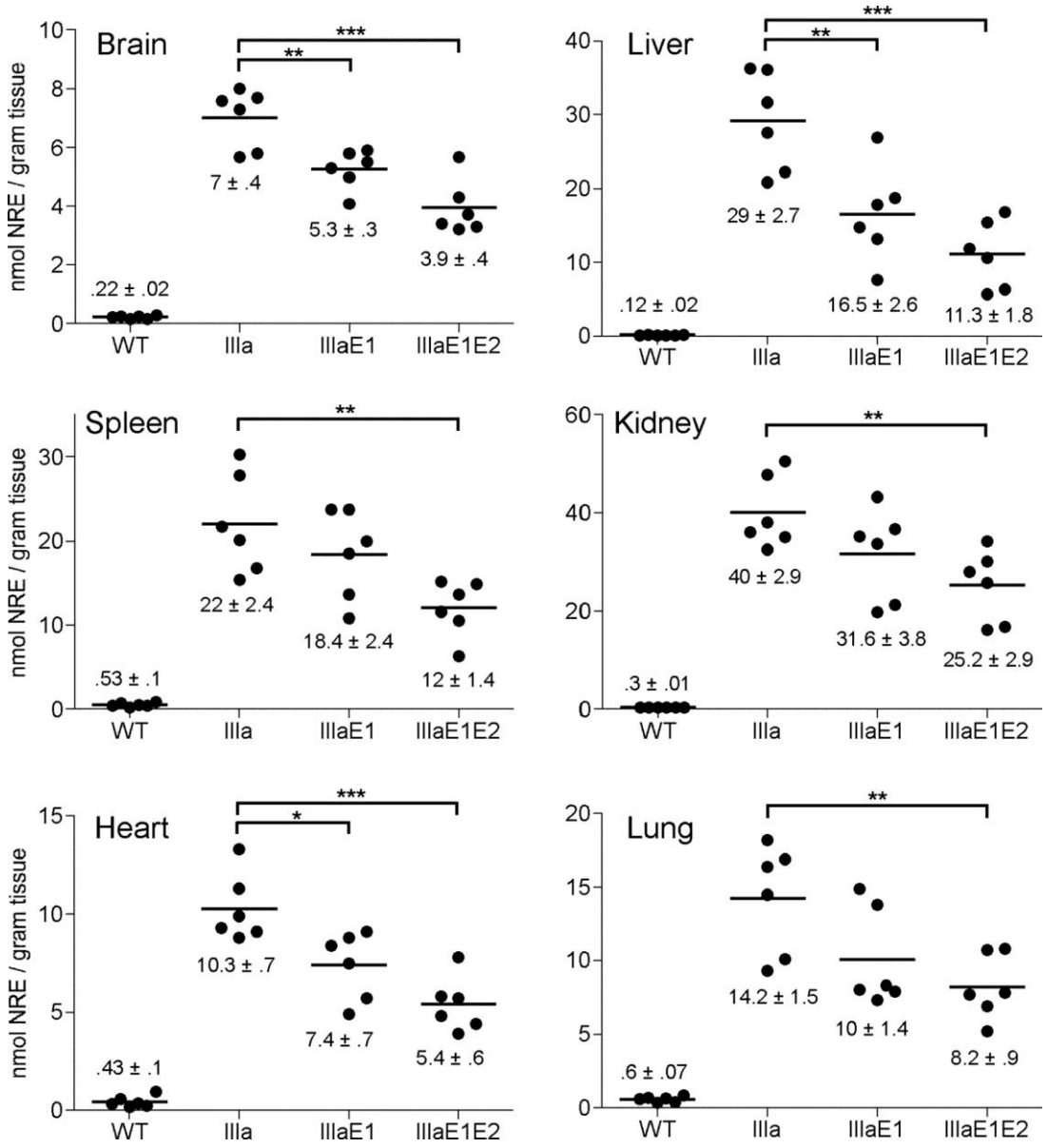
Mice:

*Sgsh*$^{-/-}$ (IIIa)
*Sgsh*$^{-/-}$*Ext1*$^{+/-}$ (IIIaE1)
*Sgsh*$^{-/-}$*Ext1*$^{+/-}$*Ext2*$^{+/-}$ (IIIaE1E2)


Core protein
Ser
Ser/Thr
XYLT1
XYLT2
B4GALT7
B3GALT6
B3GAT1,2,3
Chain initiation
Keratan sulfate II
Chondroitin/Dermatan sulfate
Heparan sulfate
CSGALNACT1,2
CHSY1,3
CHPF
CSGLCAT
EXTL1,3
EXTL2
EXT1
EXT2
Chain polymerization
ST3GAL1
ST3GAL2
B4GALT1,2,3
B3GNT1,2
DSE
CHST7,12
CHST14
CHST3,11,13
UST
GALNAC4A-6ST
NDST2
NDST1,3,4
GLCE
HS6ST1,3
HS2ST1
HS3STA1
HS3ST2,3
Chain modification
CHST1,2,4

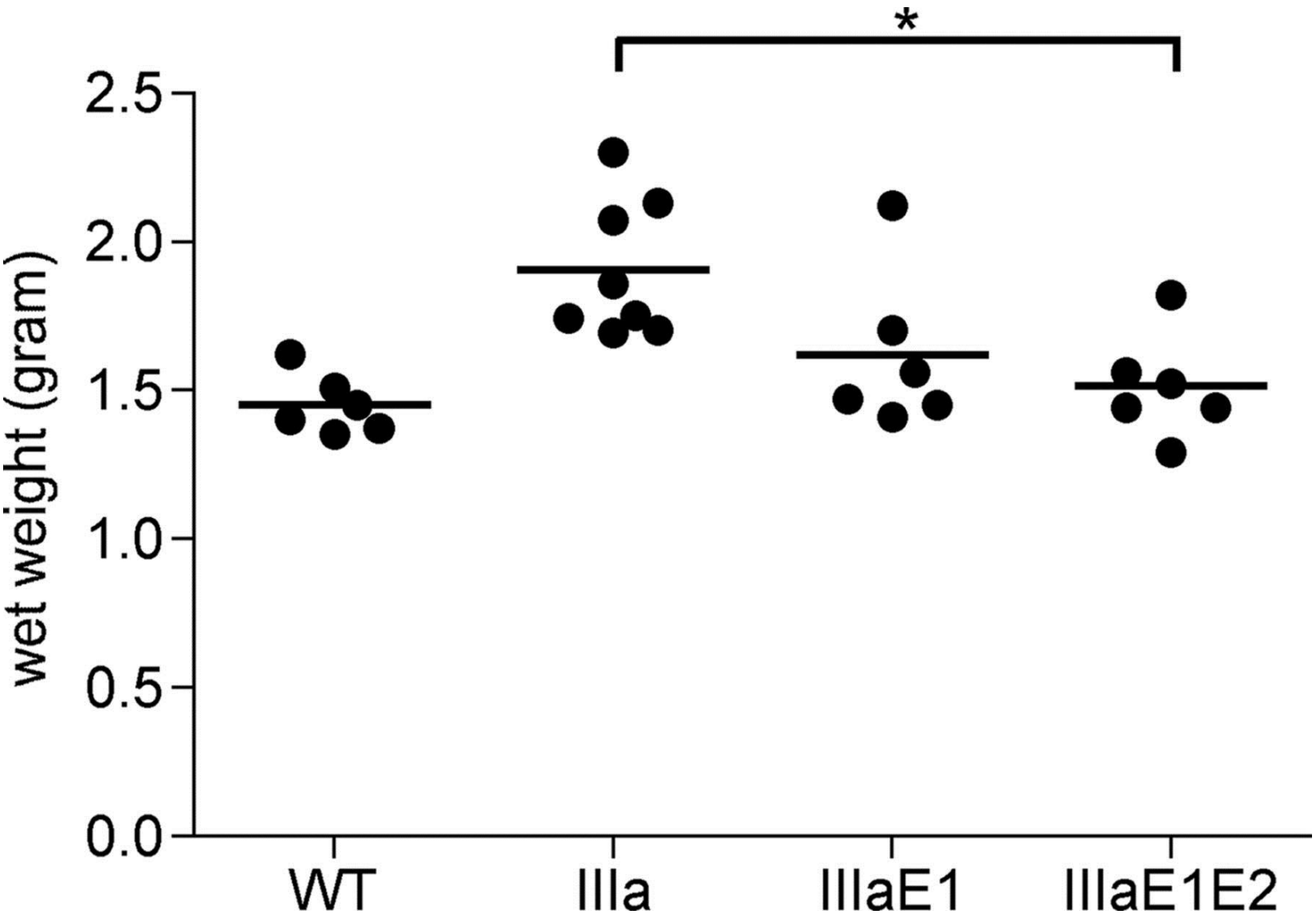
# gSRT ameliorates lysosomal storage in MPS IIIa mice

Lamanna W C et al. J. Biol. Chem. 2012;287:36283-36290



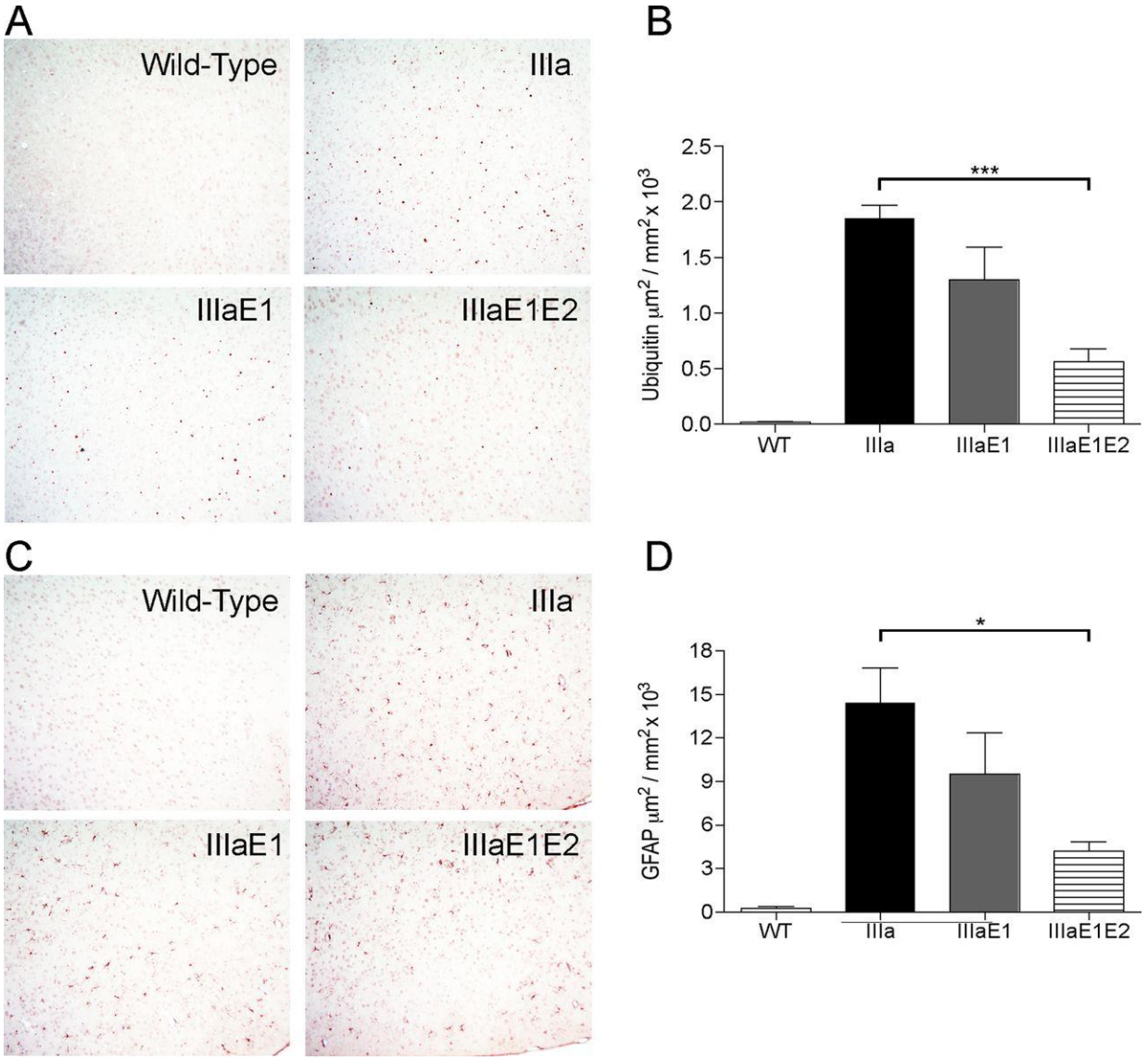
## gSRT reduces hepatomegaly in MPS IIIa mice

Lamanna W C et al. J. Biol. Chem. 2012;287:36283-36290



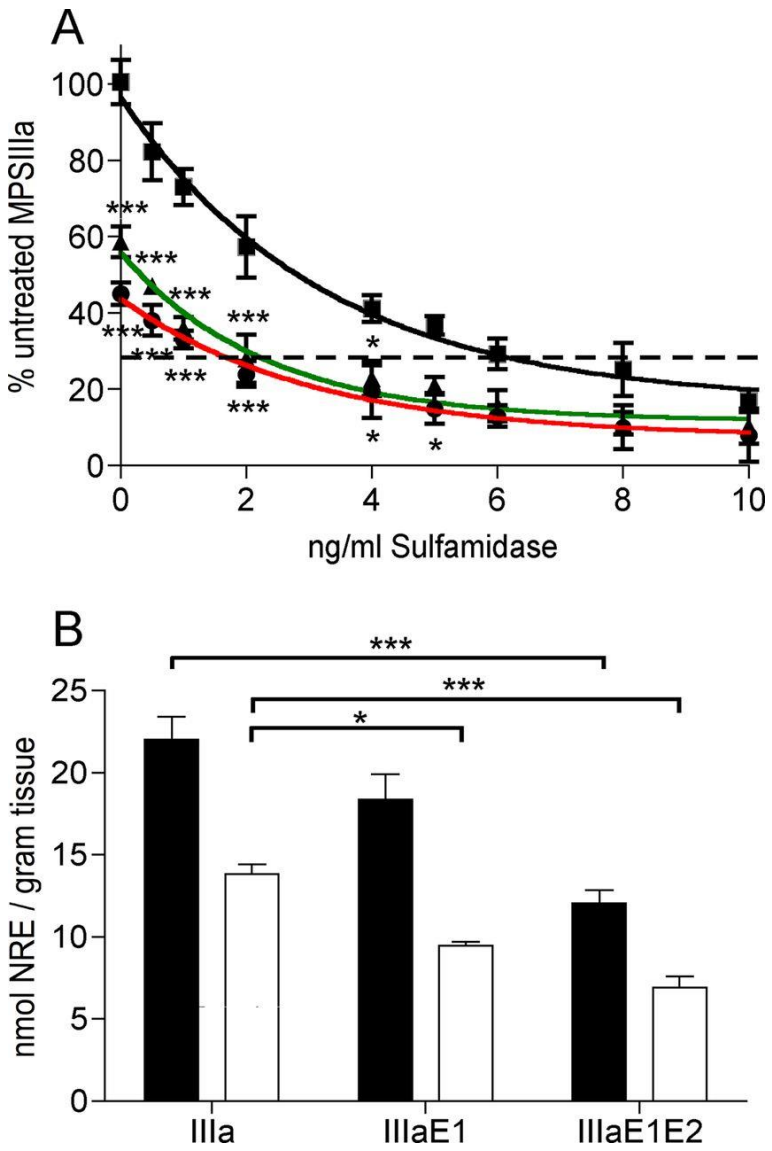
# gSRT ameliorates markers of neuropathology in MPS IIIa mice

Lamanna W C et al. J. Biol. Chem. 2012;287:36283-36290



# Ext1/Ext2 heterozygosity improves the efficacy of ERT in MPS IIIa cells and mice

Lamanna W C et al. J. Biol. Chem. 2012;287:36283-36290



# Questions

J Inherit Metab Dis
DOI 10.1007/s10545-009-9029-2

ORIGINAL ARTICLE

# Storage correction in cells of patients suffering from mucopolysaccharidoses types IIIA and VII after treatment with genistein and other isoflavones

**Audrey Arfi · Magali Richard · Christelle Gandolphe · Daniel Scherman**

Received: 1 October 2009 / Revised: 7 December 2009 / Accepted: 8 December 2009
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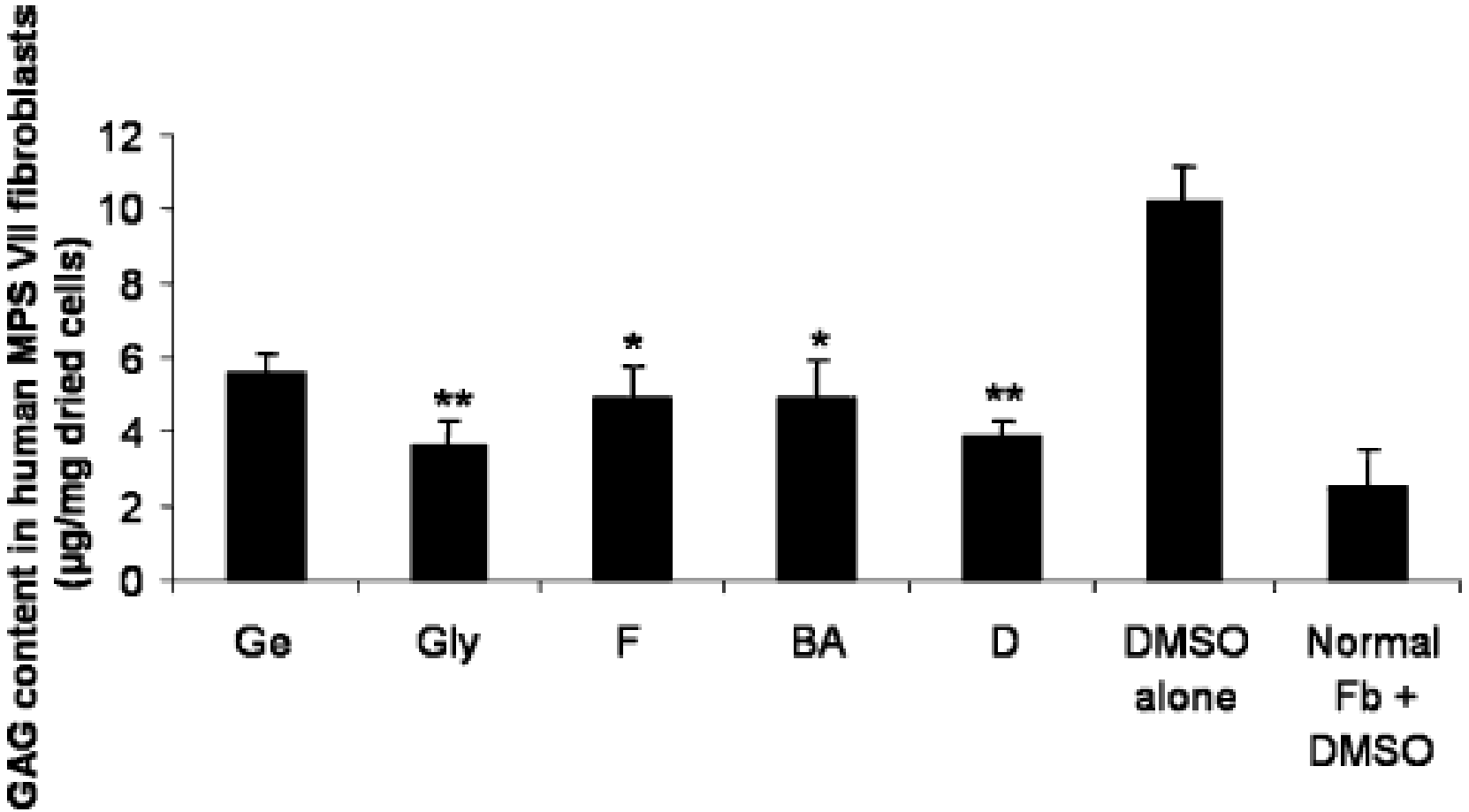
GAG content in human MPS III A fibroblasts (µg/mg dried cells)
12
10
8
6
4
2
0
*
*
*
*
*
*
Ge
Gl
F
BA
D
DMSO alone
Normal Fb + DMSO


GAG content in human MPS VII fibroblasts (µg/mg dried cells)
12
10
8
6
4
2
0
**
*
*
**
Ge
Gly
F
BA
D
DMSO alone
Normal Fb + DMSO

Molecular Genetics and Metabolism 105 (2012) 266–269

Contents lists available at SciVerse ScienceDirect

Molecular Genetics and Metabolism

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

Brief Communication

# Genistein reduces heparan sulfate accumulation in human mucolipidosis II skin fibroblasts

Takanobu Otomo *, Mohammad Arif Hossain, Keiichi Ozono, Norio Sakai

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ARTICLE INFO

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ABSTRACT

Genistein, a soy isoflavone, reduces glycosaminoglycan synthesis and its effect on mucopolysaccharidoses has been tested. In this report, we examined the effect of genistein in human mucolipidosis II skin fibroblasts *in vitro*. Heparan sulfate was accumulated within both cells and in extracellular spaces in mucolipidosis II. Genistein reduced the amount of heparan sulfate in cultured cells dose dependently and also inhibited cell growth dose dependently.

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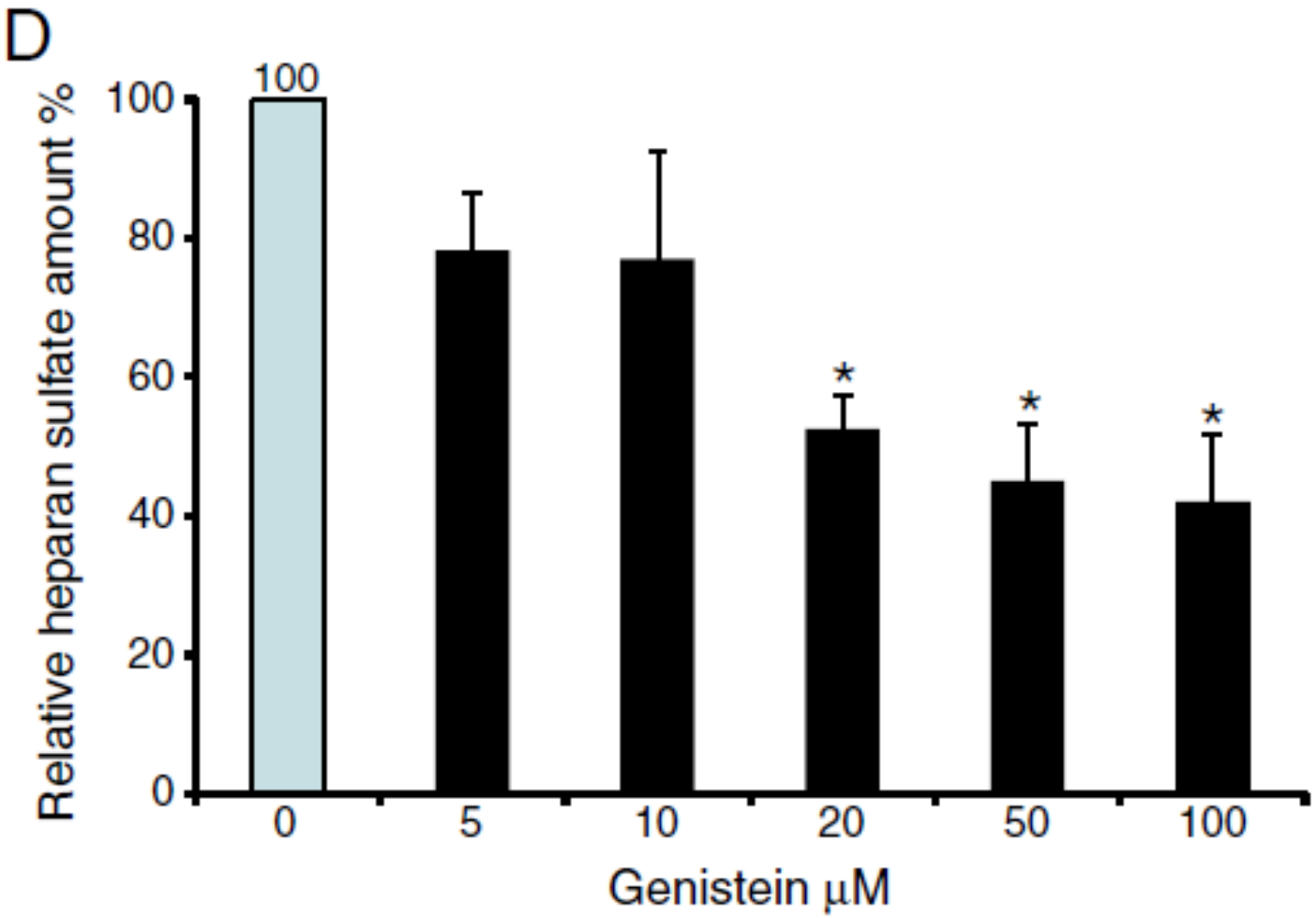
D
Relative heparan sulfate amount %
100
80
60
40
20
0
100
*
*
*
0
5
10
20
50
100
Genistein μM

BJP BRITISH JOURNAL OF PHARMACOLOGY

BPS BRITISH PHARMACOLOGICAL SOCIETY

British Journal of Pharmacology (2010), ••, ••–••
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Journal compilation © 2010 The British Pharmacological Society All rights reserved
0007-1188/10
www.brjpharmacol.org

RESEARCH PAPER

# Genistein reduces glycosaminoglycan levels in a mouse model of mucopolysaccharidosis type II

A Friso, R Tomanin, M Salvalaio and M Scarpa

*Department of Pediatrics, University of Padova, Padova, Italy*

J Inherit Metab Dis
DOI 10.1007/s10545-014-9703-x

ORIGINAL ARTICLE

# Genistein increases glycosaminoglycan levels in mucopolysaccharidosis type I cell models

**Sandra D. K. Kingma · Tom Wagemans · Lodewijk IJlst · Frits A. Wijburg · Naomi van Vlies**

Received: 10 December 2013 / Revised: 24 February 2014 / Accepted: 10 March 2014
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**Abstract** Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder characterized by diminished degradation of the glycosaminoglycans (GAGs) heparan sulfate and dermatan sulfate, which results in the accumulation of these GAGs and subsequent cellular dysfunction. Patients present with a variety of symptoms, including severe skeletal disease. Genistein has been shown previously to inhibit GAG synthesis in MPS fibroblasts, presumably through inhibition of tyrosine kinase activity of the epidermal growth factor receptor (EGFR). To determine the potentials of genistein for the treatment of skeletal disease, MPS I fibroblasts were induced into chondrocytes and osteoblasts and treated with genistein. Surprisingly, whereas tyrosine phosphorylation levels (as a measure for tyrosine kinase inhibition) were decreased in all treated cell lines, there was a 1.3 and 1.6 fold increase in GAG levels in MPS I chondrocytes and fibroblast, respectively ($p<0.05$). Sulfate incorporation in treated MPS I fibroblasts was 2.6 fold increased ($p<0.05$), indicating increased GAG synthesis despite tyrosine kinase inhibition. This suggests that GAG synthesis is not exclusively regulated through the tyrosine kinase activity of the EGFR. We hypothesize that the differences in outcomes between studies on the effect of genistein in MPS are caused by the different effects of genistein on different growth factor signaling pathways, which regulate GAG synthesis. More studies are needed to elucidate the precise signaling pathways which are affected by genistein and alter GAG metabolism in order to evaluate the therapeutic potential of genistein for MPS patients.

Communicated by: Alberto B. Burlina

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 289, NO. 24, pp. 17054–17069, June 13, 2014
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# The Phytoestrogen Genistein Modulates Lysosomal Metabolism and Transcription Factor EB (TFEB) Activation*

Received for publication, February 4, 2014, and in revised form, April 24, 2014 Published, JBC Papers in Press, April 25, 2014, DOI 10.1074/jbc.M114.555300

**Marta Moskot[‡], Sandro Montefusco[§], Joanna Jakóbkiewicz-Banecka[¶], Paweł Mozolewski[¶], Alicja Węgrzyn[‖], Diego Di Bernardo[§], Grzegorz Węgrzyn[¶], Diego L. Medina[§1], Andrea Ballabio[§**‡‡§§], and Magdalena Gabig-Cimińska[‡2]**

*From the [‡]Laboratory of Molecular Biology (affiliated with the University of Gdańsk), Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Wita Stwosza 59, 80-308 Gdańsk, Poland, the [§]High Content Screening Facility, Telethon Institute of Genetics and Medicine (TIGEM), Via P. Castellino 111, 80131 Naples, Italy, the [¶]Department of Molecular Biology, University of Gdańsk, Wita Stwosza 59, 80-308 Gdańsk, Poland, the [‖]Department of Microbiology, University of Szczecin, Felczaka 3c, 71-412 Szczecin, Szczecin, Poland, the **Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, Texas 77030, the [‡‡]Jan and Dan Duncan Neurological Research Institute, Texas Children's Hospital, Houston, Texas 77030, and [§§]Medical Genetics, Department of Pediatrics, Federico II University, Via Pansini 5, 80131 Naples, Italy*

**Background:** Genistein is a potential drug for certain inherited lysosomal disorders.
**Results:** Genistein influences molecular cross-talk in the cell responsible for lysosomal enhancement.
**Conclusion:** Genistein potentiates lysosomal metabolism by activating transcription factor EB (TFEB).
**Significance:** The explanation of genistein action offers more adequate therapeutic procedures for the treatment of some lysosomal storage diseases.

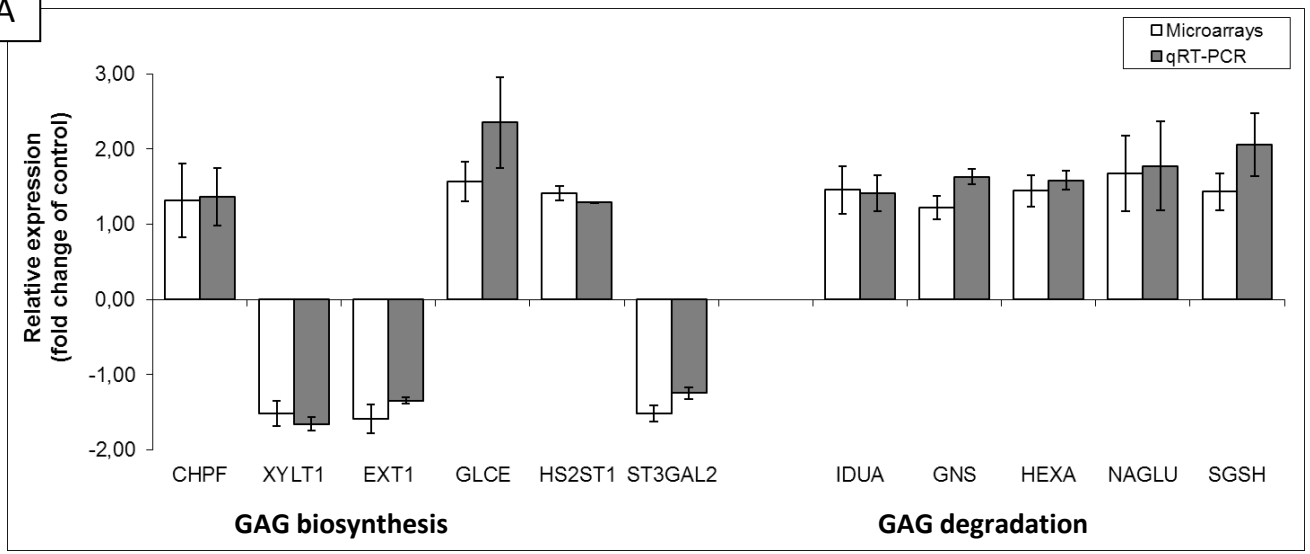
Microarray analyses using HumanHT-12 Expression BeadChip

Transcriptomes of human fibroblasts treated with genistien

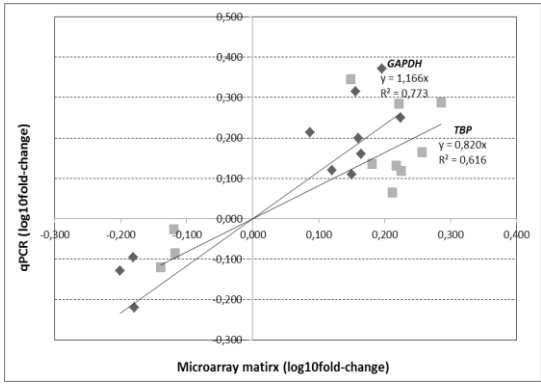
| **Gene expression modulation** | **Modulation targets** | **No. of genes** | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | **Genistein concentration [$\mu$M]** | | | | | | | | |
| | | **30** | | | **60** | | | **100** | | |
| | | **Time of exposure [h]** | | | | | | | | |
| | | **1** | **24** | **48** | **1** | **24** | **48** | **1** | **24** | **48** |
| ▲ up-regulated genes | whole genome | 7 | 12 | 25 | 1 | 110 | 155 | 6 | 231 | 291 |
| | GAG metabolism | 1 | 1 | 0 | 2 | 7 | 6 | 2 | 10 | 6 |
| | GSL metabolism | 0 | 1 | 2 | 1 | 7 | 10 | 1 | 6 | 10 |
| ▼ down-regulated genes | whole genome | 18 | 21 | 34 | 8 | 149 | 215 | 23 | 236 | 370 |
| | GAG metabolism | 0 | 0 | 0 | 0 | 4 | 1 | 0 | 4 | 3 |
| | GSL metabolism | 0 | 2 | 0 | 0 | 2 | 0 | 0 | 2 | 1 |

# Expression of genes coding for enzymes involved in GAG synthesis and degradation is response to genistein, as assessed by microarray analyses and qRT-PCR

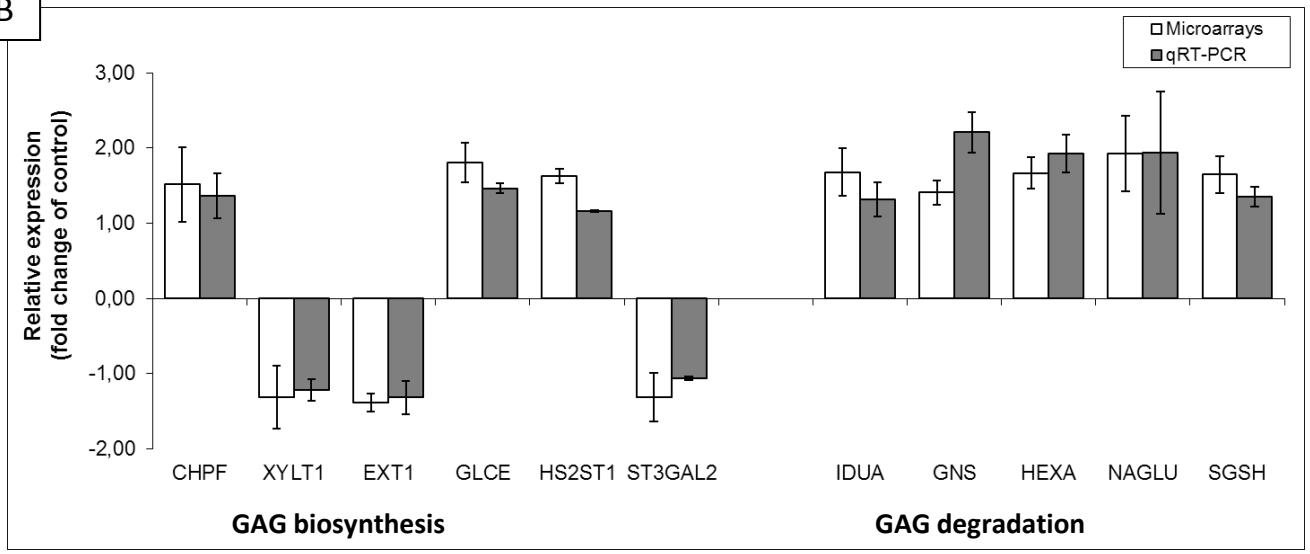
A



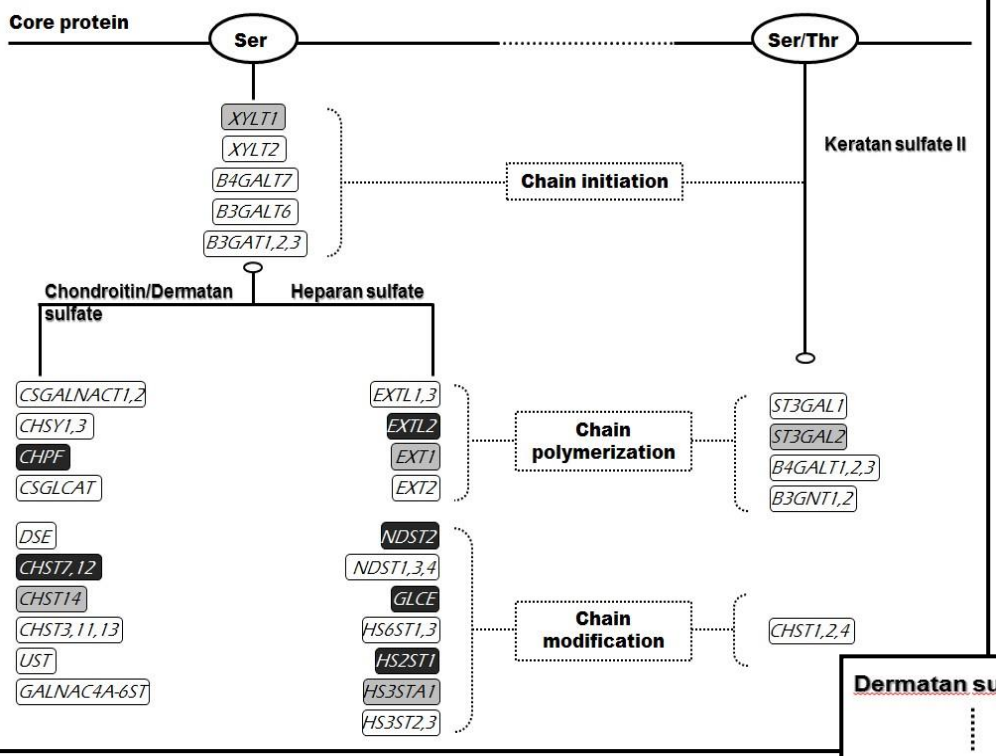

Relative to *GAPDH* as a control




B

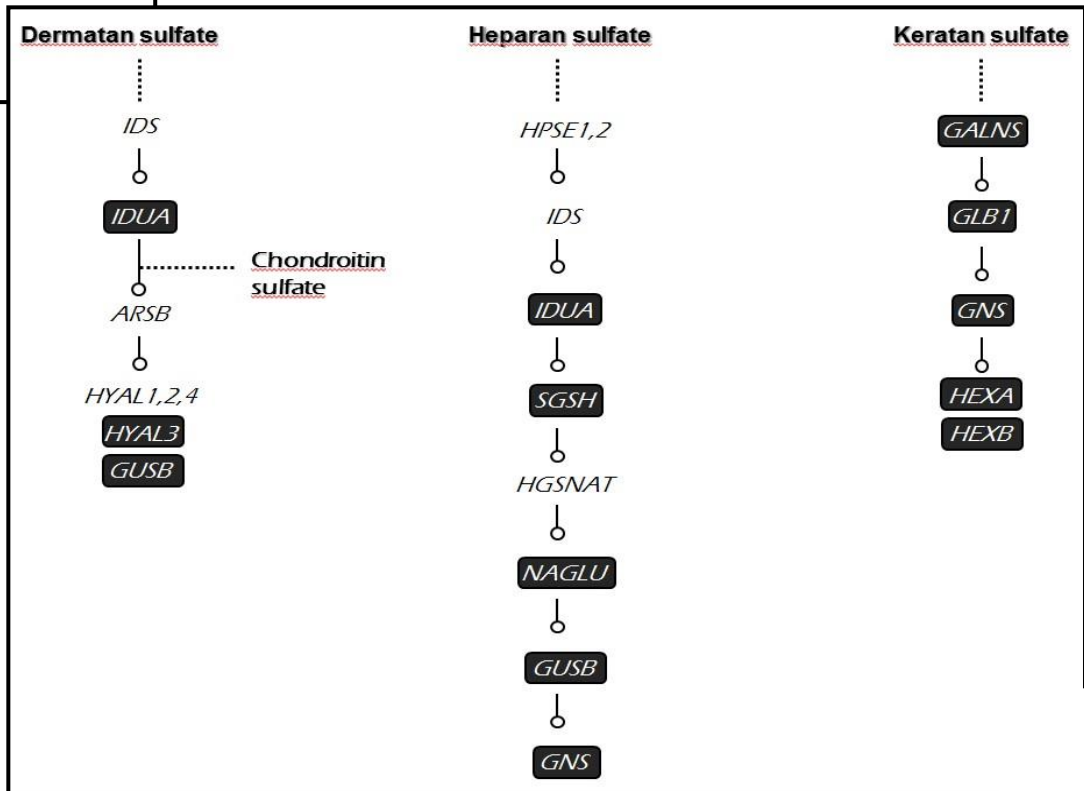



Relative to*TBP* as a control




**GAG synthesis pathways**

**GAG degradation pathways**

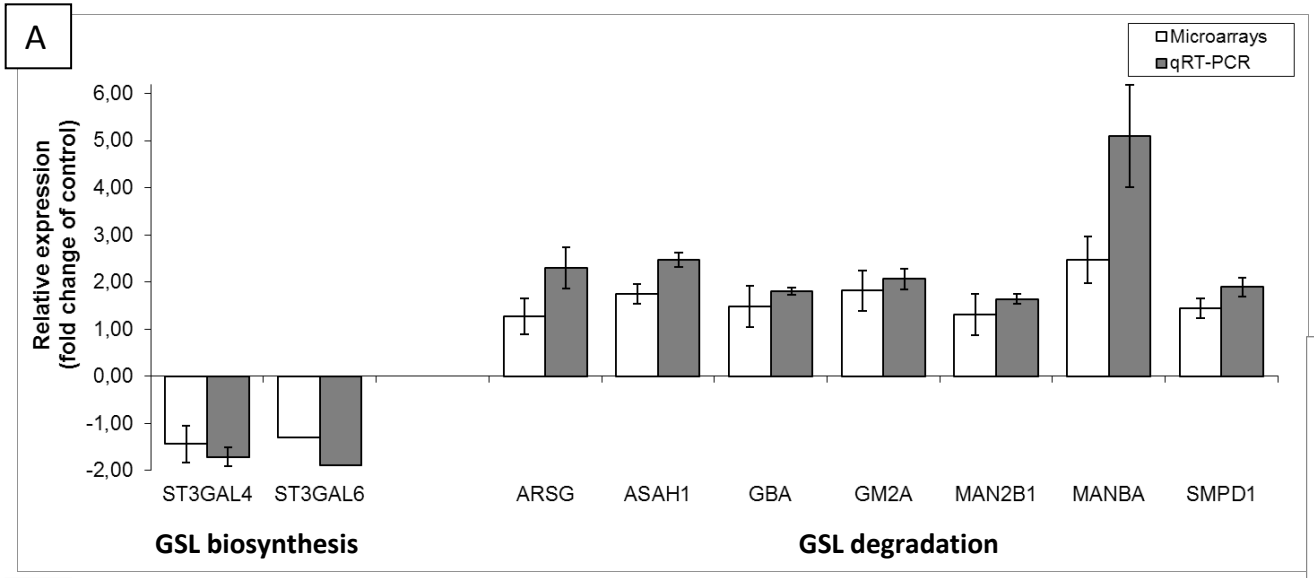


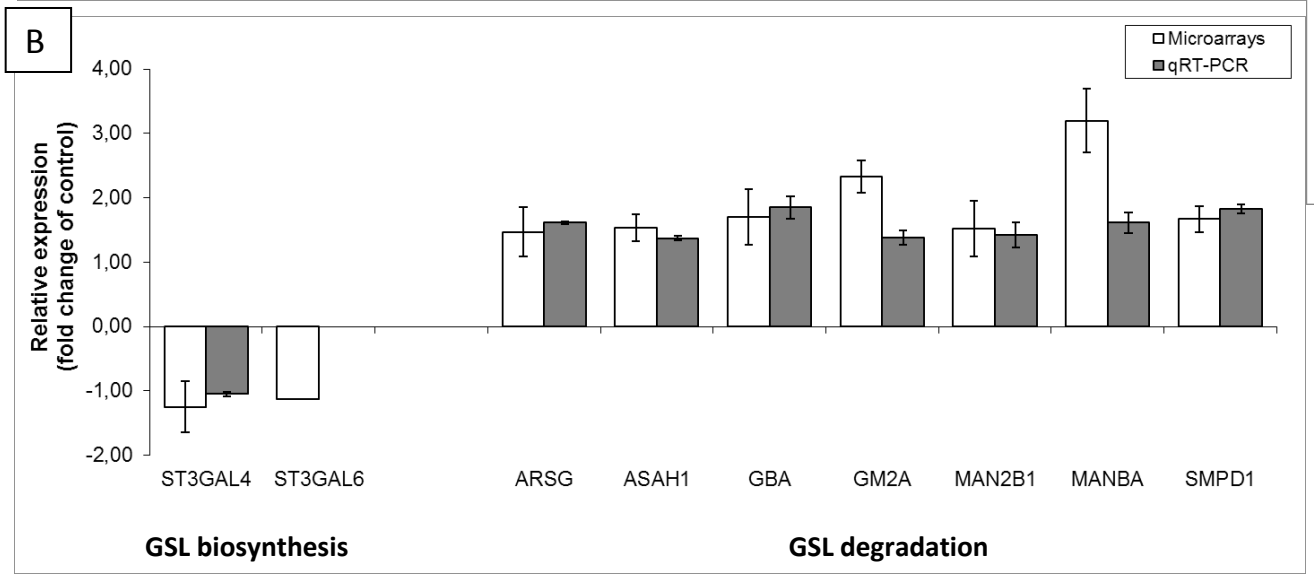

Stimulation by genistein

Inhibition by genistein

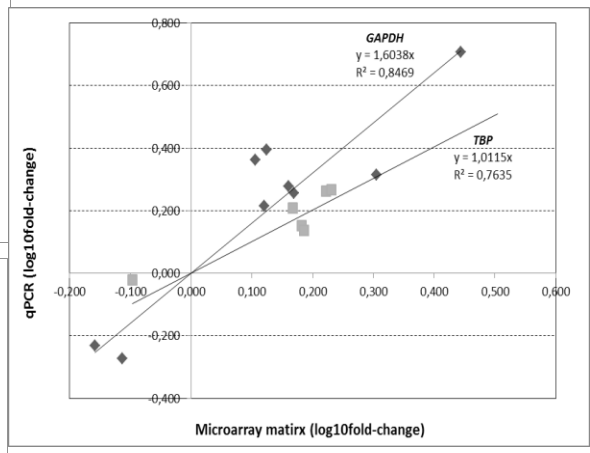
No strong effect of genistein

# Expression of genes coding for enzymes involved in glycosphingolipids' (GSL) synthesis and degradation is response to genistein, as assessed by microarray analyses and qRT-PCR



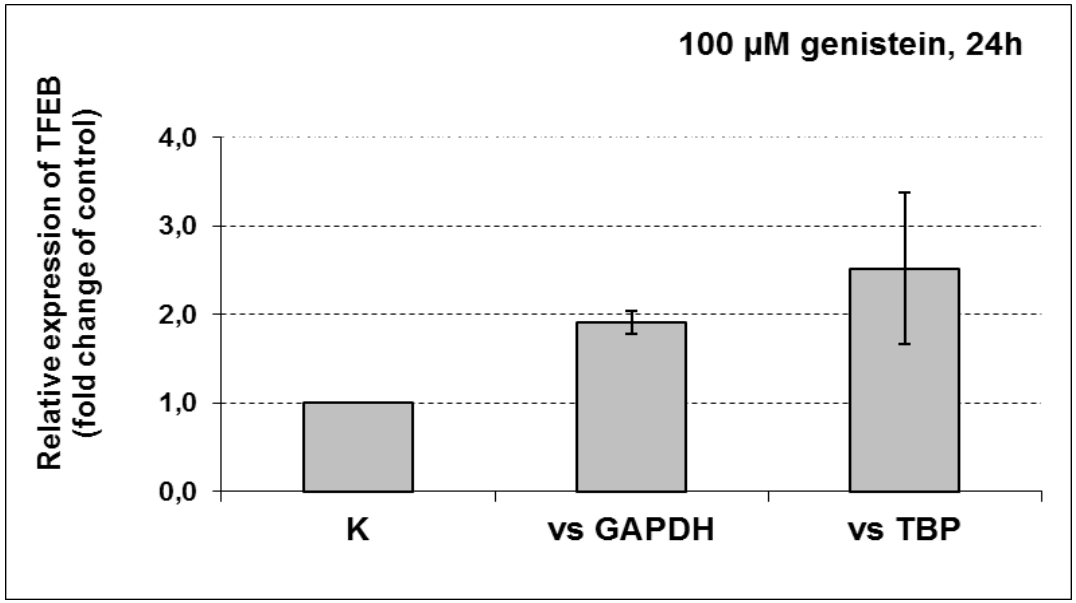




Relative to *GAPDH* as a control

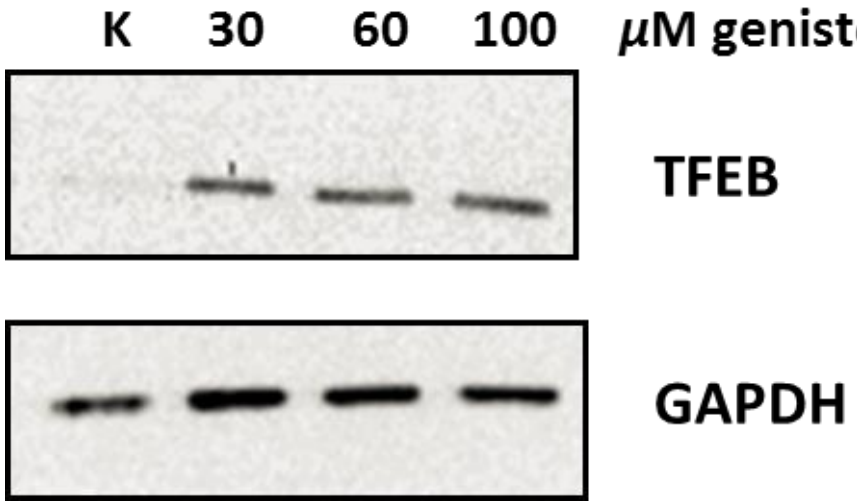



Relative to*TBP* as a control

# Expression *TFEB,* a gene coding for the master positive regulator of lysosomal biogenesis, is stimulated by genistein

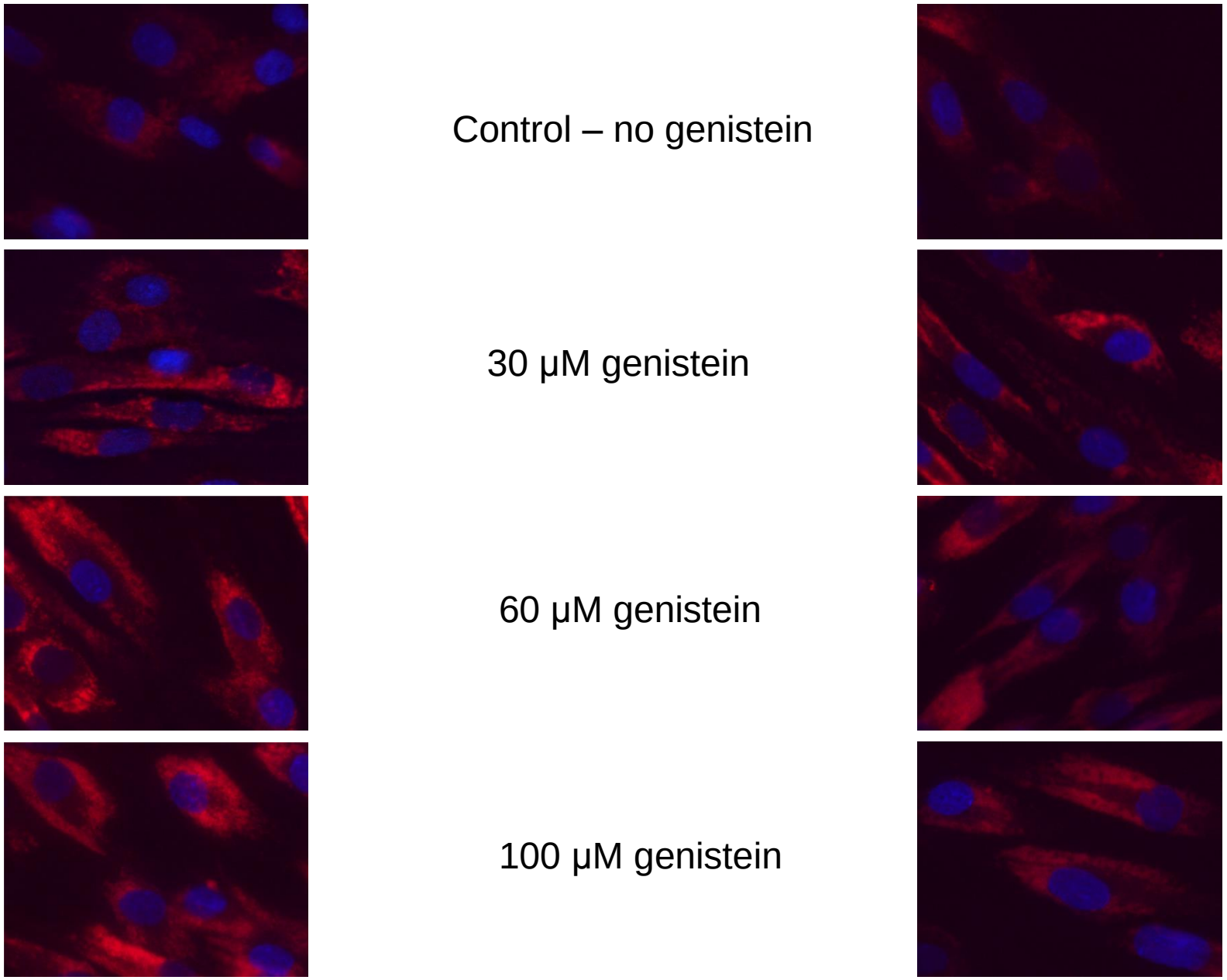



**mRNA level**




**Protein level**

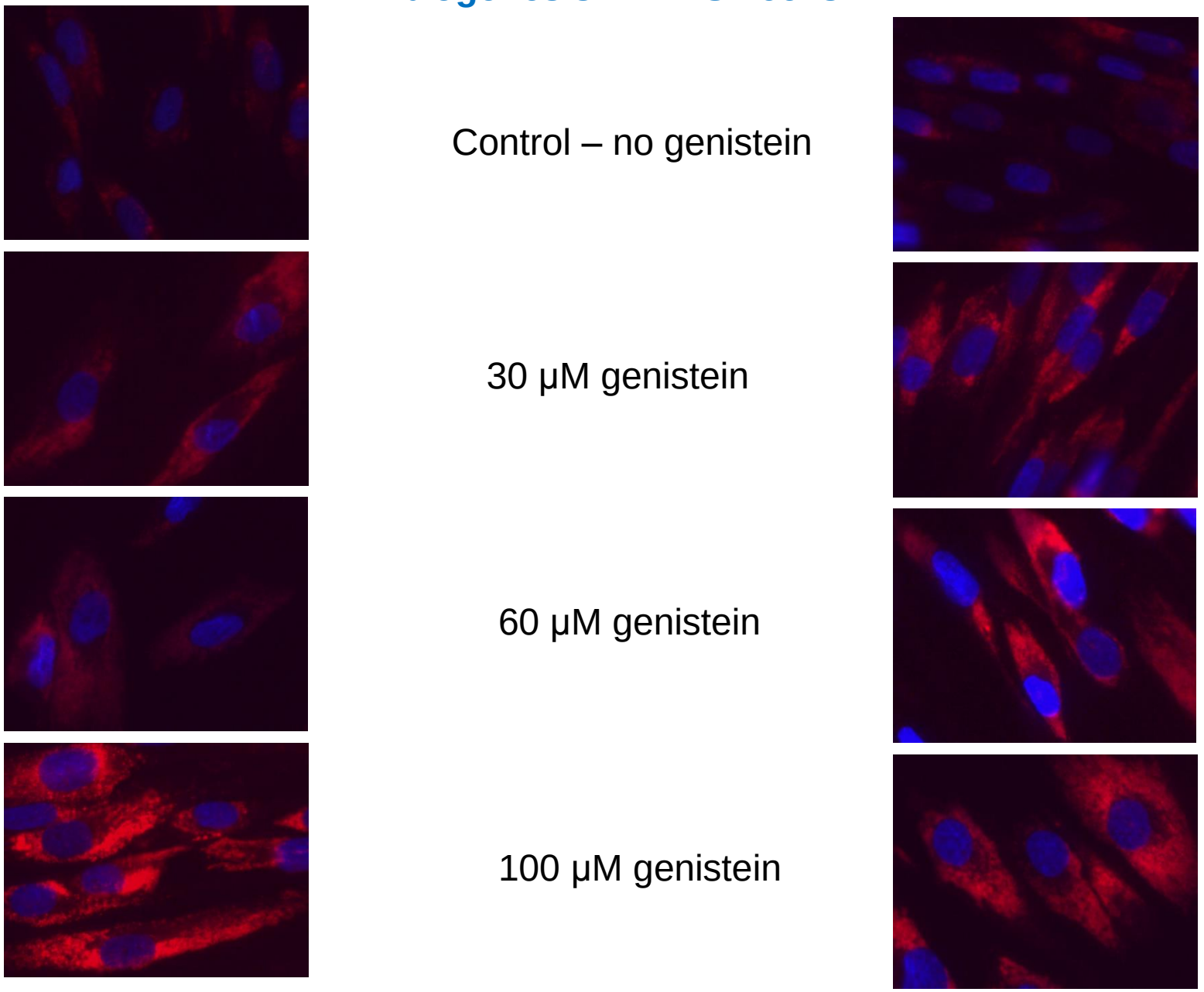
# Genistein stimulates lysosomal biogenesis in HDFa cells



# Genistein stimulates lysosomal biogenesis in MPS I cells

24 h

48h



Control – no genistein

30 μM genistein

60 μM genistein

100 μM genistein

CURRENT THERAPEUTIC RESEARCH

VOLUME 69, NUMBER 2, APRIL 2008

*Brief Report*

# Genistin-Rich Soy Isoflavone Extract in Substrate Reduction Therapy for Sanfilippo Syndrome: An Open-Label, Pilot Study in 10 Pediatric Patients

Ewa Piotrowska, MSc[1]; Joanna Jakóbkiewicz-Banecka, PhD[1,2]; Anna Tylki-Szymanska, MD, PhD, DSc[3]; Anna Liberek, MD, PhD[4]; Agnieszka Maryniak, PhD, DSc[3]; Marcelina Malinowska, MSc[1]; Barbara Czartoryska, PhD[5]; Ewa Puk, PhD[6]; Anna Kloska, MSc[1]; Tomasz Liberek, MD, PhD, DSc[7]; Sylwia Baranska, PhD[1]; Alicja Wegrzyn, PhD, DSc[2]; and Grzegorz Wegrzyn, PhD, DSc[1]

*[1]Department of Molecular Biology, University of Gdansk, Gdansk, Poland; [2]Laboratory of Molecular Biology, Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Gdansk, Poland; [3]The Children's Memorial Health Institute, Warsaw, Poland; [4]Department of Pediatrics, Children's Gastroenterology and Oncology, Medical University of Gdansk, Gdansk, Poland; [5]Department of Genetics, Institute of Psychiatry and Neurology, Warsaw, Poland; [6]Biofarm, Poznan, Poland; and [7]Department of Nephrology, Transplantation, and Internal Medicine, Medical University of Gdansk, Gdansk, Poland*

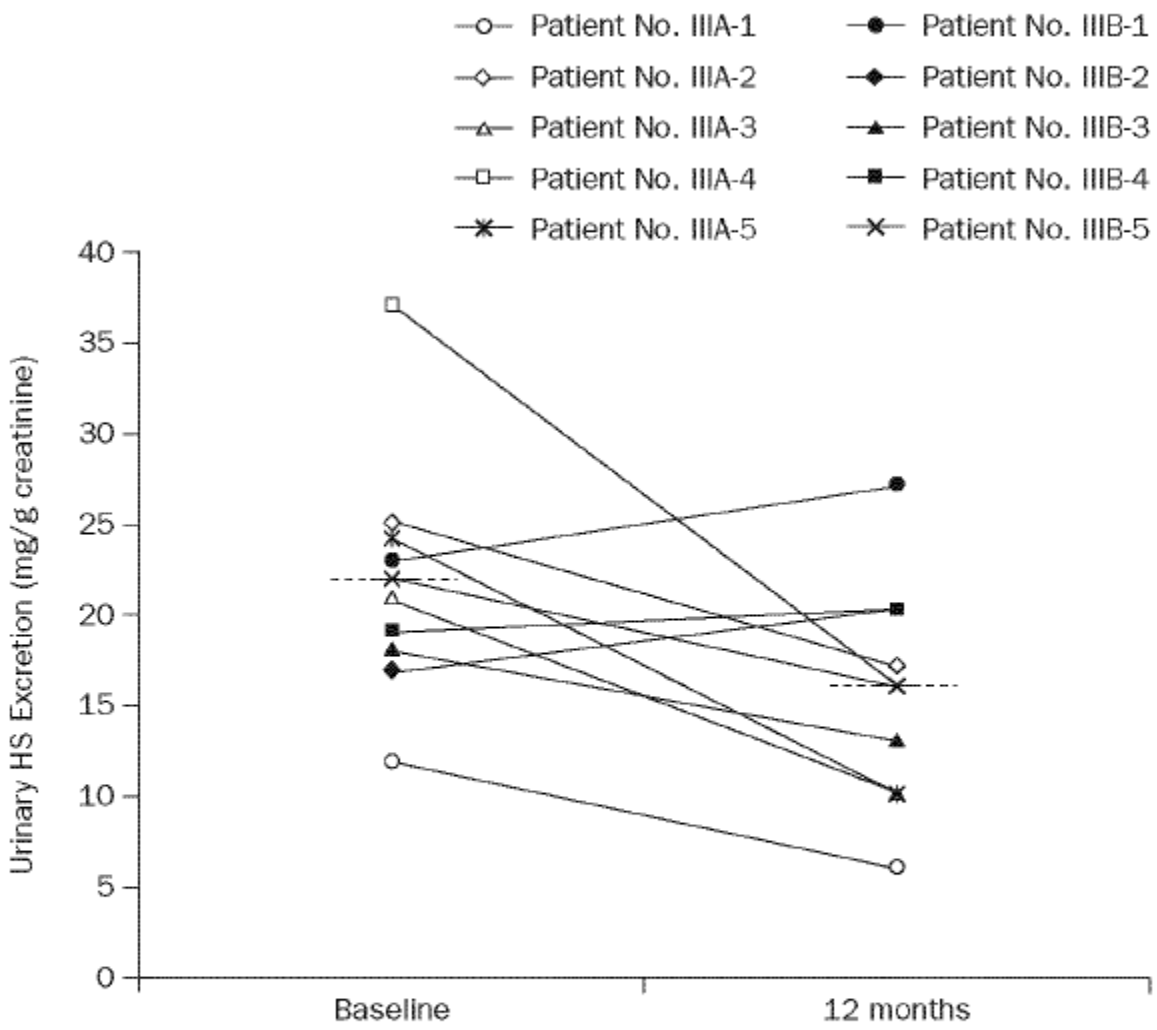



**Figure 1. Urinary heparan sulfate (HS) concentrations in patients with mucopolysaccharidosis (MPS) IIIA or IIIB treated with a genistin-rich isoflavone extract. (Median values are shown by the dashed horizontal bars at baseline and 12 months.) *P* = 0.028 versus baseline. Significant changes were found in patients IIIA-1, IIIA-2, IIIA-3, IIIA-4, IIIA-5, IIIB-3, and IIIB-5.**

**Table II. Hair morphology assessed using electron microscopy and cognitive function assessed using the Brief Assessment Examination (BAE) at baseline and after 12 months of treatment with genistin-rich isoflavone extract.[21–23]**

| | Hair Morphology Score* | | BAE Score† | |
|---|---|---|---|---|
| Patient No. | Baseline | 12 Months | Baseline | 12 Months |
| IIIA-1 | 1 | 0‡ | 19 | 23‡ |
| IIIA-2 | 1 | 0‡ | 14 | 19‡ |
| IIIA-3 | 2 | 1‡ | 36 | 36 |
| IIIA-4 | 2 | 1‡ | 0 | 4‡ |
| IIIA-5 | 3 | 1‡ | 11 | 17‡ |
| IIIB-1 | 1 | 1 | 9 | 12‡ |
| IIIB-2 | 1 | 1 | 33 | 36‡ |
| IIIB-3 | 2 | 1‡ | 12 | 12 |
| IIIB-4 | 3 | 2‡ | 4 | 6‡ |
| IIIB-5 | 3 | 1‡ | 27 | 32‡ |

*Scale: 0 = normal to 5 = most abnormal.
†Scale: 0 = no contact with the tested child to 52 = normal score for properly developed child at the age of 3 years.
‡$P = 0.012$ versus baseline.

J Inherit Metab Dis (2011) 34:1039–1044
DOI 10.1007/s10545-011-9342-4

ORIGINAL ARTICLE

# Genistein supplementation in patients affected by Sanfilippo disease

**Verónica Delgadillo · Maria del Mar O'Callaghan · Rafael Artuch · Raquel Montero · Mercedes Pineda**

Received: 25 January 2011 / Revised: 14 April 2011 / Accepted: 15 April 2011 / Published online: 10 May 2011
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**Table 3** Clinical and biochemical results at baseline and after 12 months of treatment

| | Baseline | | | 12 months | | |
|---|---|---|---|---|---|---|
| | Mean | Standard deviation | Reference values | Mean | Standard deviation | Wilcoxon test |
| GAGs (mg/mmol creat.) | 27.54 | 13.94 | (0.36–6.4) 6[a] | 35.30 | 19.48 | n.s |
| CoQ10 (μmol/L) | 0.42 | 0.16 | (0.41–1.12) 0.70 | 0.36 | 0.11 | n.s. |
| Cholesterol (mmol/L) | 3.8 | 0.75 | (2.47–5.20) 2.73 | 4.07 | 0.99 | n.s. |
| Vitamin E (μmol/L) | 20.16 | 5.89 | (13.4–36.4) 23 | 27.67 | 7.73 | n.s. |
| Disability score | 9.5 | 6.27 | | 10.85 | 6.2[b] | $P=0.012$ |

*GAG* Glycosaminoglycan, *n.s.* not significant

[a] GAG reference values are average values for 6 year old

[b] Significant worsening in disability scores was observed after 12 months of therapy

**Table 4** Hair morphology determined with electron microscopy at baseline and after 12 months of treatment with genistein-rich isoflavone extract

| Patient | Hair morphology score[a] | |
|---|---|---|
| | Baseline | After first year |
| III A - 1 | 2 | 0 |
| III A - 2 | 1 | 0 |
| III A - 3 | 3 | 1 |
| III A - 4 | 2 | 0 |
| III A - 5 | 1 | 0 |
| III A - 6 | 3 | 1 |
| III A - 7 | 0 | 0 |
| III A - 8 | 4 | 2 |
| III A - 9 | 2 | 0 |
| III A - 10 | 1 | 0 |
| III B - 2 | 2 | 0 |
| III C - 1 | 3 | 0 |
| III C - 3 | 0 | 0 |
| III C - 5 | 2 | 0 |
| III C - 6 | 2 | 0 |

[a] Scores range from 0 (normal) to 5 (most abnormal)

CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

# Improvement in the Range of Joint Motion in Seven Patients With Mucopolysaccharidosis Type II During Experimental Gene Expression-Targeted Isoflavone Therapy (GET IT)

**Jolanta Marucha,[1] Anna Tylki-Szymańska,[1] Joanna Jakóbkiewicz-Banecka,[2] Ewa Piotrowska,[2] Anna Kloska,[2] Barbara Czartoryska,[3] and Grzegorz Węgrzyn[1]***

[1]Department of Metabolic Diseases, The Children's Memorial Health Institute, Warsaw, Poland

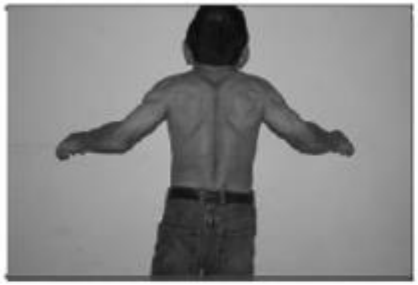
[2]Department of Molecular Biology, University of Gdańsk, Gdańsk, Poland

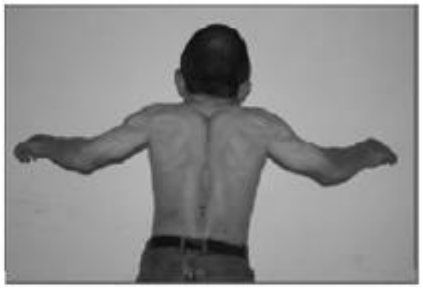
[3]Department of Genetics, Institute of Psychiatry and Neurology, Warsaw, Poland

Received 25 May 2010; Accepted 23 April 2011

Baseline

26 weeks of genistein administration





.Active shoulder abduction

Passive shoulder abduction

Active shoulder flexion

Passive shoulder flexion

# Genistein in Sanfilippo Disease: A Randomized Controlled Crossover Trial

Jessica de Ruijter, MD,[1] Marlies J. Valstar, MD, PhD,[1] Magdalena Narajczyk, PhD,[2] Grzegorz Wegrzyn, PhD,[2] Wim Kulik, PhD,[3] Lodewijk IJlst, BASc,[3] Tom Wagemans, BASc,[3] Willem M. van der Wal, PhD,[4] and Frits A. Wijburg, MD, PhD[1]

**Objective:** Sanfilippo disease (mucopolysaccharidosis type III [MPS III]) is a rare neurodegenerative metabolic disease caused by a deficiency of 1 of the 4 enzymes involved in the degradation of heparan sulfate (HS), a glycosaminoglycan (GAG). Genistein has been proposed as potential therapy but its efficacy remains uncertain. We aimed to determine the efficacy of genistein in MPS III.

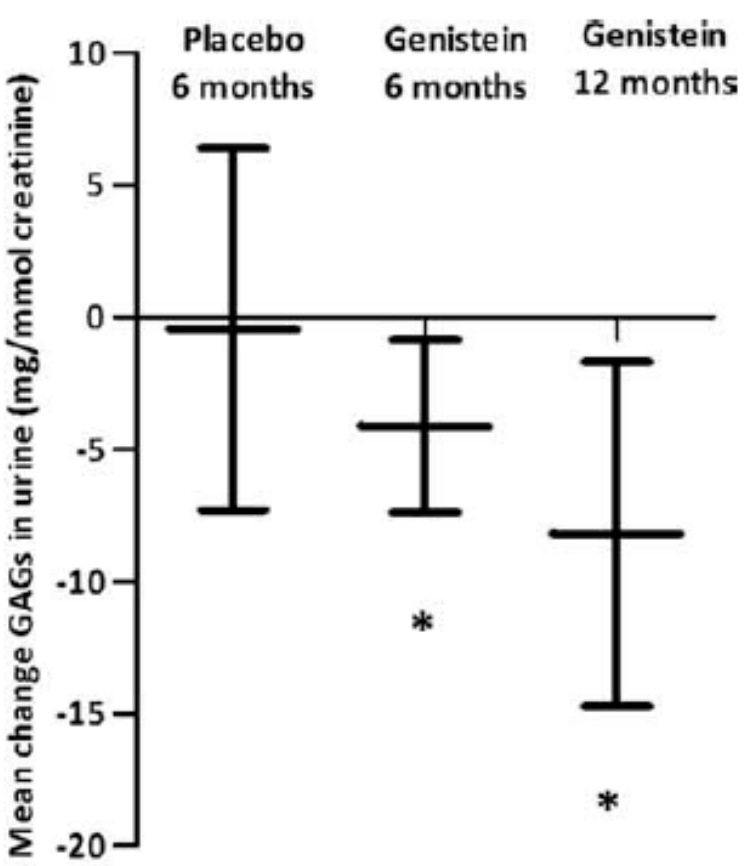
**Methods:** Thirty patients were enrolled. Effects of genistein were determined in a randomized, crossover, placebo-controlled intervention with a genistein-rich soy isoflavone extract (10mg/kg/day of genistein) followed by an open-label extension study for patients who were on genistein during the last part of the crossover.

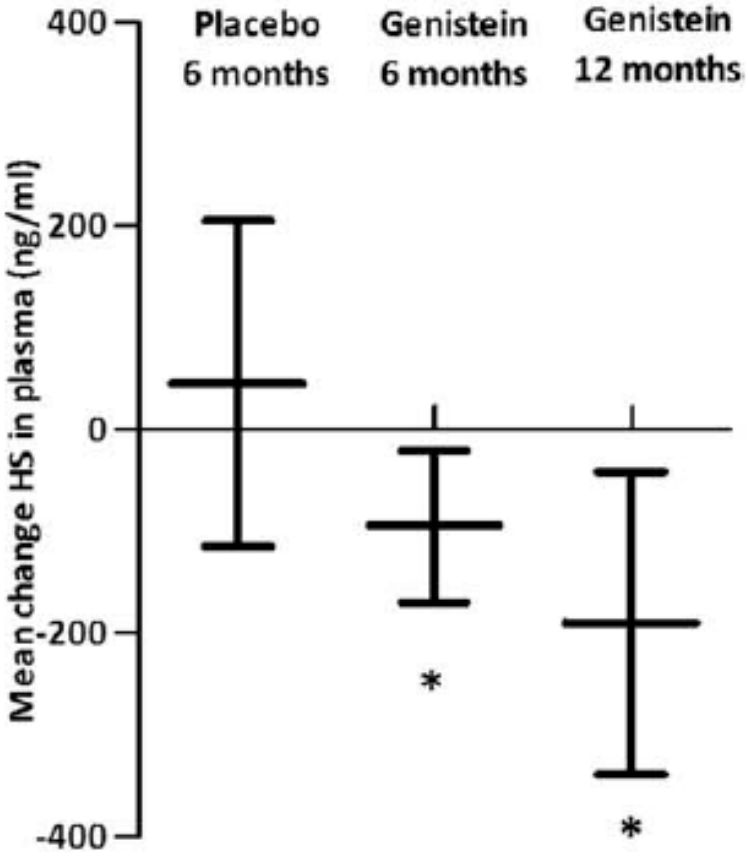
**Results:** Genistein resulted in a significant decrease in urinary excretion of total GAGs ($p = 0.02$, slope −0.68mg GAGs/mmol creatinine/mo) and in plasma concentrations of HS ($p = 0.01$, slope −15.85ng HS/ml/mo). No effects on total behavior scores or on hair morphology were observed. Parents or caregivers could not predict correctly during which period of the crossover a patient was on genistein.

**Interpretation:** Genistein at 10mg/kg/day effectively reduces urinary excretion of GAGs and plasma HS concentration in patients with MPS III. However, the absolute reduction in GAGs and in HS is small and values after 12 months of treatment remain within the range as observed in untreated patients. No clinical efficacy was detected. Substantially higher doses of genistein might be more effective as suggested by recent studies in animal models.

ANN NEUROL 2012;71:110–120

# Genistein decreases levels of total GAGs and heparan sulfate in MPS III patients



JIMD Reports
DOI 10.1007/8904_2011_87

RESEARCH REPORT

# The Use of Elevated Doses of Genistein-Rich Soy Extract in the Gene Expression-Targeted Isoflavone Therapy for Sanfilippo Disease Patients

**Věra Malinová • Grzegorz Węgrzyn • Magdalena Narajczyk**

Received: 11 May 2011 / Revised: 26 June 2011 / Accepted: 25 August 2011 / Published online: 11 December 2011
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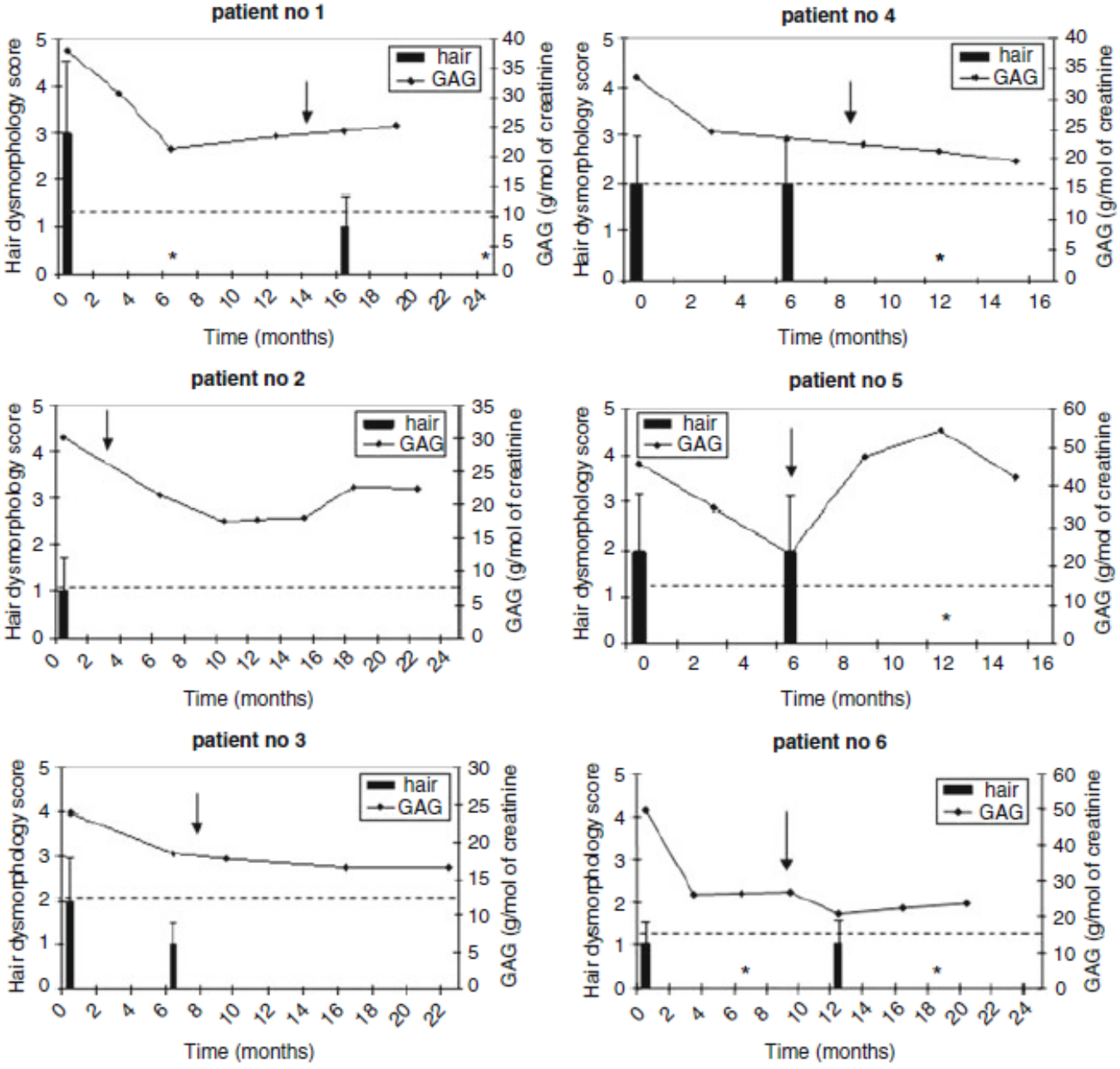
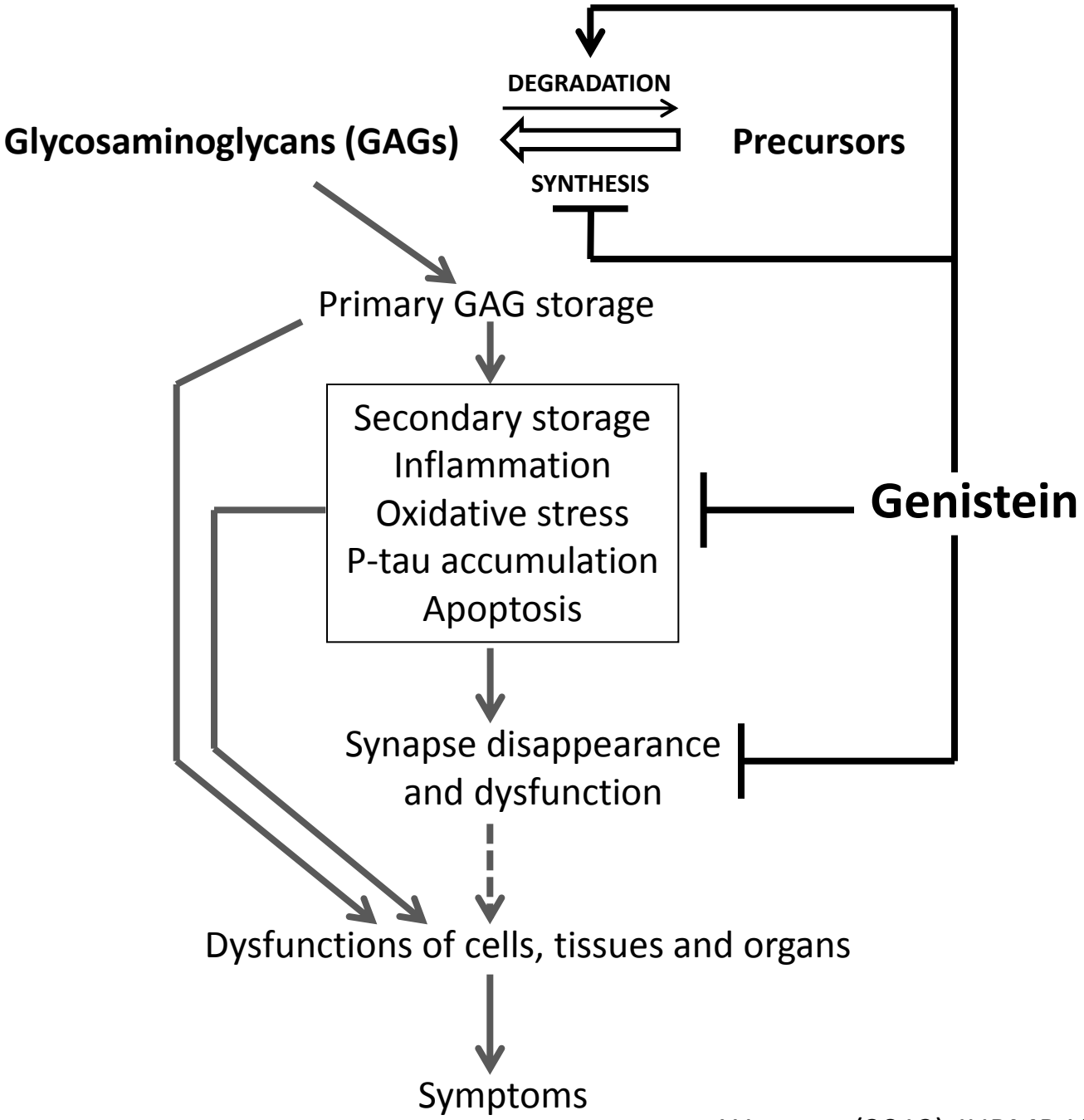

patient no 1
Hair dysmorphology score
GAG (g/mol of creatinine
hair
GAG
Time (months)
patient no 4
Hair dysmorphology score
GAG (g/mol of creatinine
hair
GAG
Time (months)
patient no 2
Hair dysmorphology score
GAG (g/mol of creatinine
hair
GAG
Time (months)
patient no 5
Hair dysmorphology score
GAG (g/mol of creatinine
hair
GAG
Time (months)
patient no 3
Hair dysmorphology score
GAG (g/mol of creatinine
hair
GAG
Time (months)
patient no 6
Hair dysmorphology score
GAG (g/mol of creatinine
hair
GAG
Time (months)




Wegrzyn (2012) *IUBMB Life* **64:** 307-315

# Further Possibilities

Molecular Genetics and Metabolism 109 (2013) 382–385

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Molecular Genetics and Metabolism

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

# High dose genistein aglycone therapy is safe in patients with mucopolysaccharidoses involving the central nervous system

Katherine H. Kim [a,b], Charlotte Dodsworth [a], Andrea Paras [a,b], Barbara K. Burton [a,b,*]

[a] Ann & Robert H. Lurie Children's Hospital of Chicago, 225 E. Chicago Ave, Chicago, IL 60611, USA
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Mucopolysaccharidosis
Sanfilippo
Genistein
Treatment

ABSTRACT

Genistein (4,5,7-trihydroexyisoflavone), a soy derived isoflavone, has been proposed as a substra
therapy in patients with mucopolysaccharidoses (MPS) disorders with central nervous system
based on studies in cultured fibroblasts demonstrating that this agent inhibits glycosaminoglyc
Several studies have reported treatment of MPS III patients with low dose genistein (5–15 mg/
no serious adverse effects and variable neurocognitive outcomes. Mice with MPS IIIB treated wi
(160 mg/kg/day) genistein exhibited a significant decrease in heparan sulfate accumulation and neuroi
in the brain and improvement of the behavioral phenotype. No study to date has been performed us
genistein treatment in MPS patients. We initiated an open label study to assess the safety of high d
treatment in MPS patients with neurological impairment. Twenty-two eligible patients were tre
12 months with pure synthetic genistein at a dose of 150 mg/kg/day. Safety labs, urine GAG levels,

# Sanfilippo Clinical Trial

## A Phase III, Double Blinded, Randomised, Placebo Controlled Clinical Tr
## High Dose Oral Genistein Aglycone in Patients with Sanfilippo Synd
## (Mucopolysaccharidosis III A, B and C) - GENiSIS2013.

Released November 26 2013

*Stem Cell & Neurotherapies Group, Centre for Genomic Medicine, University of Manchester*
*Willink Biochemical Genetics Unit, Centre for Genomic Medicine, St Mary's Hospital, C*
*Manchester University Hospitals NHS Foundation Trust*

We will soon be recruiting for a phase III, double blinded, randomised, placebo controlled clinical t
high dose oral genistein aglycone in Sanfilippo diseases (MPSIIIA, B and C). This is funded by th
society for Mucopolysaccharide Diseases, The National MPS society and the GEM appeal in a grant
Brian Bigger and sponsored by the Central Manchester Universities Hospitals NHS Foundation Trus
expect to begin recruiting in early 2014, primarily from within the UK.

The trial will be regulated by the UK MHRA, performed at the NIHR/Wellcome Trust Clinical Res
facility in Manchester using GMP grade genistein aglycone. The trial will be one year placebo cont
with one year open label extension with robust efficacy and safety endpoints.

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ORIGINAL PAPER

# Effects of flavonoids on glycosaminoglycan synthesis: implications for substrate reduction therapy in Sanfilippo disease and other mucopolysaccharidoses

**Anna Kloska · Joanna Jakóbkiewicz-Banecka · Magdalena Narajczyk · Zyta Banecka-Majkutewicz · Grzegorz Węgrzyn**

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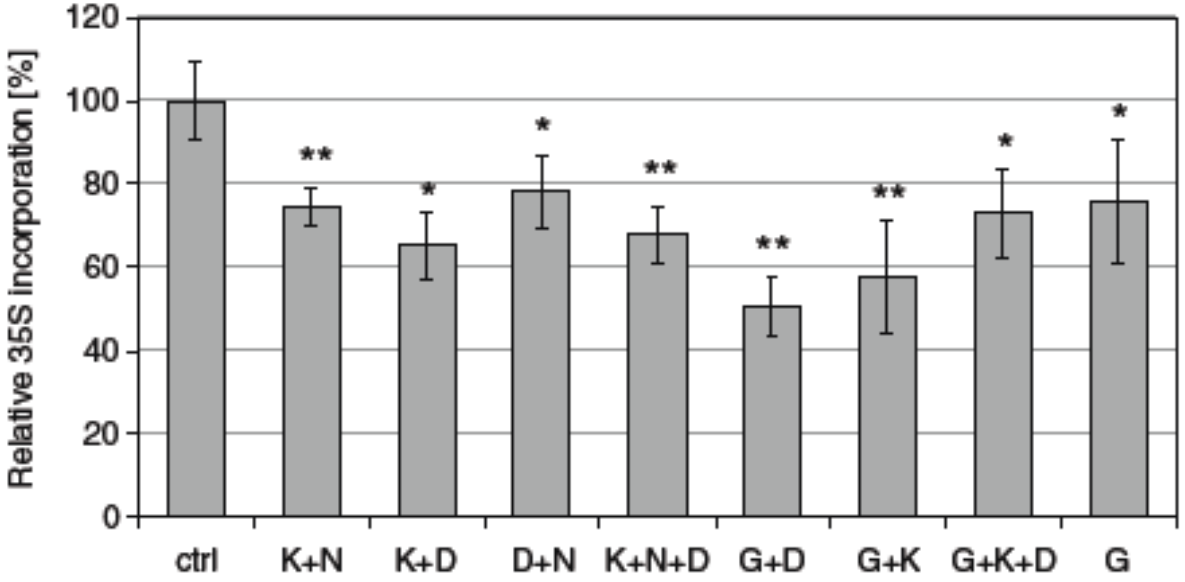


**Fig. 3** Effects of mixtures of natural flavonoids (K—kaempferol, N—naringenin, D—daidzein, G—genistein, at 10 μM concentration each) on kinetics of glycosaminoglycan synthesis in fibroblasts. Relative $^{35}S$ incorporation into GAGs after 3-day exposure to mixtures of various flavonoids is presented. Labeling was conducted for 24 h with 20 μCi/ml $H_2[^{35}S]O_4$. Radioactivity of incorporated $^{35}S$ was measured in a scintillation counter, calculated per DNA amount [dpm/ng DNA], and expressed as the percentage of control (ctrl = cell culture treated with 0.05% dimethylformamide). The results presented are average values obtained for three different cell *lines with bars* indicating standard deviation. Statistical analysis was performed by using the *t*-Student two-tailed test. Values of $p<0.05$ (*) or $p<0.01$ (**) are indicated

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RESEARCH Open Access

# Synthetic genistein derivatives as modulators of glycosaminoglycan storage

Anna Kloska[1], Magdalena Narajczyk[2], Joanna Jakóbkiewicz-Banecka[1], Grzegorz Grynkiewicz[3], Wiesław Szeja[4], Magdalena Gabig-Cimińska[1,5] and Grzegorz Węgrzyn[1*]

**Table 3 Effect of synthetic derivatives of genistein at 30 μM concentration on the number of different lysosomal structures in MPS IIIA and MPS IIIB fibroblasts**

| Compound | Number of structures per 100 $\mu m^2$ of cellular cross section ± SD | | | |
|---|---|---|---|---|
| | lamellar | complexed | amorphous | total number |
| | | **MPS IIIA** | | |
| None | 0.40 ± 0.17 | 0.35 ± 0.35 | 0.38 ± 0.27 | 1.13 ± 0.50 |
| IFG-032 | 0.14 ± 0.08 * | 0.22 ± 0.11 | 0.17 ± 0.16 * | 0.53 ± 0.19 * |
| IFG-034 | 0.17 ± 0.09 * | 0.27 ± 0.14 | 0.17 ± 0.09 * | 0.61 ± 0.20 * |
| IFG-036 | 0.19 ± 0.14 * | 0.28 ± 0.16 | 0.22 ± 0.11 | 0.73 ± 0.31 * |
| IFG-038 | 0.32 ± 0.16 | 0.27 ± 0.13 | 0.21 ± 0.13 | 0.80 ± 0.24 * |
| IFG-066 | 0.13 ± 0.10 * | 0.16 ± 0.08 | 0.18 ± 0.19 * | 0.48 ± 0.17 * |
| IFG-071 | 0.28 ± 0.13 | 0.26 ± 0.09 | 0.25 ± 0.11 | 0.79 ± 0.22 * |
| IFG-072 | 0.20 ± 0.10 * | 0.28 ± 0.10 | 0.34 ± 0.17 | 0.82 ± 0.23 |
| | | **MPS IIIB** | | |
| None | 0.44 ± 0.18 | 0.14 ± 0.10 | 0.17 ± 0.12 | 0.75 ± 0.31 |
| IFG-032 | 0.17 ± 0.11 * | 0.10 ± 0.07 | 0.12 ± 0.08 | 0.40 ± 0.20 * |
| IFG-034 | 0.20 ± 0.16 * | 0.06 ± 0.05 | 0.15 ± 0.08 | 0.41 ± 0.16 * |
| IFG-036 | 0.25 ± 0.21 * | 0.12 ± 0.11 | 0.15 ± 0.18 | 0.50 ± 0.34 |
| IFG-038 | 0.21 ± 0.12 * | 0.10 ± 0.10 | 0.09 ± 0.09 | 0.41 ± 0.18 * |
| IFG-066 | 0.15 ± 0.10 * | 0.11 ± 0.14 | 0.08 ± 0.06 | 0.34 ± 0.23 * |
| IFG-071 | 0.07 ± 0.07 * | 0.07 ± 0.06 | 0.13 ± 0.09 | 0.26 ± 0.13 * |
| IFG-072 | 0.11 ± 0.12 * | 0.16 ± 0.08 | 0.14 ± 0.10 | 0.41 ± 0.20 * |

Asterisks (*) indicate statistically significant differences (one-way ANOVA with Tukey's multiple comparisions as a post-hoc test, $p < 0.05$) relative to control MPS IIIA and MPS IIIB cells (None) where no tested compound was added into culture medium.

# Microarray analyses using HumanHT-12 Expression BeadChip

## Transcriptomes of human fibroblasts treated with genistien, kaempferol, daidzein, and their combinations

| Gene expression modulation | Modulation targets | No. of genes | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Genistein [$\mu$M] | | | | | | Kaempferol [$\mu$M] | | | | | | Genistein + Kaempferol [$\mu$M] | | Daidzein [$\mu$M] | | | | Genistein + Daidzein [$\mu$M] | |
| | | 30 | | 60 | | 100 | | 30 | | 60 | | 100 | | 30 + 30 | | 60 | | 100 | | 30 + 30 | |
| | | Time of exposure [h] | | | | | | | | | | | | | | | | | | | |
| | | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 | 24 | 48 |
| ▲ up-regulated genes | whole genome | 12 | 25 | 110 | 155 | 231 | 291 | 286 | 284 | 220 | 538 | 421 | 812 | 209 | 496 | 75 | 287 | 26 | 112 | 38 | 463 |
| | GAG metabolism | 1 | 0 | 7 | 6 | 10 | 6 | 1 | 7 | 1 | 8 | 6 | 13 | 6 | 10 | 0 | 5 | 0 | 4 | 1 | 6 |
| | GSL metabolism | 1 | 2 | 7 | 10 | 6 | 10 | 6 | 12 | 5 | 13 | 11 | 19 | 8 | 20 | 1 | 5 | 3 | 10 | 4 | 15 |
| ▼ down-regulated genes | whole genome | 21 | 34 | 149 | 215 | 236 | 370 | 266 | 221 | 120 | 439 | 279 | 697 | 154 | 835 | 27 | 232 | 31 | 443 | 46 | 139 |
| | GAG metabolism | 0 | 0 | 4 | 1 | 4 | 3 | 4 | 0 | 3 | 3 | 5 | 5 | 2 | 3 | 1 | 3 | 1 | 7 | 2 | 2 |
| | GSL metabolism | 2 | 0 | 2 | 0 | 2 | 1 | 4 | 1 | 2 | 3 | 6 | 6 | 3 | 5 | 0 | 2 | 0 | 3 | 1 | 2 |

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