



High-Throughput Screen Fails to Identify Compounds That Enhance Residual Enzyme Activity of Mutant *N*-Acetyl- α -Glucosaminidase in Mucopolysaccharidosis Type IIIB

O. L. M. Meijer · P. van den Biggelaar · R. Ofman ·
F. A. Wijburg · N. van Vlies

Received: 02 March 2017 / Revised: 19 June 2017 / Accepted: 24 July 2017 / Published online: 24 August 2017
© Society for the Study of Inborn Errors of Metabolism (SSIEM) 2017

Abstract Background: In the severe neurodegenerative disorder mucopolysaccharidosis type IIIB (MPSIIIB or Sanfilippo disease type B), deficiency of the lysosomal enzyme *N*-acetyl- α -glucosaminidase (NAGLU) results in accumulation of heparan sulfate. Patients present with a severe, rapidly progressing phenotype (RP) or a more attenuated, slowly progressing phenotype (SP). In a previous study, residual NAGLU activity in fibroblasts of SP patients could be increased by culturing at 30°C, probably as a result of improved protein folding and lysosomal targeting under these conditions. Chaperones are molecules which influence protein folding and could therefore have therapeutic potential in SP MPSIIIB patients. Here we studied the effects of 1,302 different compounds on residual NAGLU activity in SP MPSIIIB patient

fibroblasts including 1,280 approved compounds from the Prestwick Chemical Library.

Methods: Skin fibroblasts of healthy controls, an SP MPSIIIB patient (homozygous for the temperature sensitive mutation p.S612G) and an RP MPSIIIB patient (homozygous for the p.R297* mutation and non-temperature sensitive), were used. A high-throughput assay for measurement of NAGLU activity was developed and validated, after which 1,302 different molecules were tested for their potential to increase NAGLU activity.

Results: None of the compounds tested were able to enhance NAGLU activity.

Conclusions: This high-throughput screen failed to identify compounds that could enhance residual activity of mutant NAGLU in fibroblasts of SP MPSIIIB patients with temperature sensitive mutations. To therapeutically simulate the positive effect of lower temperatures on residual NAGLU activity, first more insight is needed into the mechanisms underlying this temperature dependent increase.

Communicated by: Roberto Giugliani, MD, PhD

Electronic supplementary material: The online version of this chapter (doi:10.1007/8904_2017_51) contains supplementary material, which is available to authorized users.

O.L.M. Meijer · F.A. Wijburg (✉) · N. van Vlies
Department of Pediatric Metabolic Diseases, Emma Children's Hospital and Amsterdam Lysosome Center "Sphinx", Academic Medical Center, Amsterdam, The Netherlands
e-mail: o.l.meijer@amc.uva.nl; f.a.wijburg@amc.uva.nl; naomi.van.vlies@intravacc.nl

O.L.M. Meijer · P. van den Biggelaar · R. Ofman · N. van Vlies
Laboratory of Genetic Metabolic Diseases, Department of Clinical Chemistry, Academic Medical Center, Amsterdam, The Netherlands
e-mail: o.l.meijer@amc.uva.nl; peggyvandenbiggelaar@gmail.com; r.ofman@amc.uva.nl; naomi.van.vlies@intravacc.nl

N. van Vlies
Intravacc, Institute for Translational Vaccinology, Bilthoven, The Netherlands
e-mail: naomi.van.vlies@intravacc.nl

Introduction

In mucopolysaccharidosis type IIIB (MPSIIIB or Sanfilippo disease type B; OMIM#: 252920), deficiency of the lysosomal enzyme *N*-acetyl- α -glucosaminidase (NAGLU; EC: 3.2.1.50) results in accumulation of the glycosaminoglycan (GAG) heparan sulfate (Muenzer 2011). Patients generally present between the age of 1 and 4 years with a delay in neurocognitive development, predominantly affecting speech and language skills, which is followed by a

progressive neurocognitive decline accompanied by behavioral problems (Valstar et al. 2010). There is a wide spectrum of disease severity, ranging from a severe, rapidly progressing phenotype (RP) to a more attenuated, slowly progressing phenotype (SP). Whereas RP patients often die in their late teenage years or early adulthood, patients with an SP phenotype may show a stable developmental impairment for years (Moog et al. 2007; Valstar et al. 2010). No disease modifying treatment is yet available.

Recently, we showed that culturing skin fibroblasts of MPSIIIB patients with an SP phenotype at 30°C significantly increased residual NAGLU activity, probably due to improved protein folding, decreased degradation, and improved targeting to the lysosome (Meijer et al. 2016). Chaperones are molecules that could induce comparable effects and may be considered as potential therapeutic agents for SP MPSIIIB patients. Molecular chaperones, including the heat shock proteins, are endogenous chaperones that play an important role in protein stabilization and are key players in the intracellular protein quality control system (Hartl et al. 2011). Chemical chaperones, on the other hand, are exogenous compounds that stimulate protein folding by nonspecific modes of action (Engin and Hotamisligil 2010; Cortez and Sim 2014), whereas pharmacological chaperones stabilize proteins by more specific binding as they act as ligand to the enzyme or selectively bind a particular native conformation of the protein (Parenti 2009). The use of pharmacological chaperones has been investigated for many diseases affecting protein folding, including LSDs, and several are now in clinical trials (Hollak and Wijburg 2014; Parenti et al. 2015).

Suitable candidates for chaperone therapy in MPSIIIB are 2-acetamido-1,2-dideoxyribose (2AcDNJ) and 6-acetamido-6-deoxycastanospermine, since they were found to be potent inhibitors of purified human NAGLU and its bacterial homolog (Zhao and Neufeld 2000; Ficko-Blean et al. 2008). Another compound of interest is glucosamine. Treatment of cultured fibroblasts from MPS IIC patients (OMIM#: 252930) with glucosamine partially restored the activity of the deficient enzyme heparan acetyl-CoA:alpha-glucosaminide *N*-acetyltransferase (HGSNAT; EC:2.3.1.78). This could also be the case for MPSIIIB, as NAGLU binds glucosamine residues at the non-reducing end of the GAG chain (Feldhammer et al. 2009; Matos et al. 2014).

Here we aimed to investigate the effects of known chemical and pharmacological chaperones on residual enzyme activity in a MPSIIIB fibroblast cell line in which residual enzyme activity can be increased by culturing at low temperature. Also, we investigated the effect of the 1,280 approved compounds from the Prestwick Chemical Library, which have all proven their safety in humans.

Material and Methods

Cell Culture

Cultured skin fibroblasts of healthy controls, a MPSIIIB patient with an SP phenotype, homozygous for the temperature sensitive missense mutation p.S612G, and of a MPSIIIB patient homozygous for the p.R297* mutation conveying an RP phenotype and previously demonstrated not to be temperature sensitive, were selected for validation of the assay and subsequent compound screen (Meijer et al. 2016). Fibroblasts were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% Fetal Bovine Serum (FBS) and 100 U/mL penicillin, 100 µg/mL streptomycin, and 250 µg/mL amphotericin at 37°C (unless otherwise stated) in a humidified atmosphere containing 5% CO₂. Before adding FBS to the medium, bovine NAGLU was inactivated by incubation of FBS at 65°C for 35 min. All cell lines were found negative for mycoplasma contamination.

NAGLU Activity Assay

NAGLU activity in protein homogenates of skin fibroblasts was measured according to our previously described method (Meijer et al. 2016). Since this method is unsuitable for screening of a large number of compounds, an assay suitable for high-throughput screening was designed, based on the method described by Mauri et al. (2013). Fluorogenic 4-methylumbelliferyl-2-acetamido-2-deoxy- α -D-glucopyranoside (4MU- α -GlcNAc) (Moscerdam, Oegstgeest, The Netherlands) was used as substrate and dissolved to the required concentration in a 0.1 M citrate 0.2 M phosphate buffer pH 4.3. The assay was started by adding 50 µL reaction mixture (12.5 µL 4MU- α -GlcNAc, 37 µL 0.1 M citrate 0.2 M phosphate buffer pH 3.85 and 0.5 µL 10% Triton-X100) to each well which was incubated at 37°C for different time periods. The reaction was stopped by adding 150 µL 0.2 M sodium carbonate buffer pH 10.5. Fluorescence of released 4-methylumbelliferone was measured with a Fluostar Optima Microplate Reader (BMG Labtech, Ortenberg, Germany), using an excitation and emission wavelength of 360 nm and 450 nm, respectively. NAGLU activity was calculated using a calibration curve of 4-methylumbelliferone (Glycosynth Ltd., Warrington, Cheshire, UK).

Compound Screen

Cells were harvested by trypsinization, counted using a ZTM series Coulter Counter (Beckman Coulter Inc., Brea,

California, United States) and diluted in culture medium to the required concentrations. For each cell line, 100 μ L cell suspension per well was plated in black, clear bottom 96-well plates (Greiner Bio-One, Kremsmünster, Austria). Next day, culture medium was replaced with 200 μ L culture medium containing one of the small compounds described below and incubated for 5 days following our standard protocol. After 5 days incubation, plates were washed three times with phosphate buffered saline (PBS) and NAGLU activity was measured as described above.

Chemicals

Taurine, D-arginine, L-homoarginine hydrochloride, saccharose, trimethylamine *N*-oxide (TMAO), dimethyl sulfoxide (DMSO $\geq 99.9\%$), ambroxol, D-glucosamine hydrochloride, *N*-acetylglucosamine, trichostatin A (TSA), bortezomib, and ursodeoxycholic acid (UDCA) were all purchased from Sigma-Aldrich (St. Louis, MO, USA). L-arginine monohydrochloride, trehalose and 4-phenylbutyrate (4-PBA) were from Merck (Darmstadt, Germany), β -alanine from BDH (Analytical Chemicals, VWR International, Radnor, PA, USA), glycerol and betaine were from Arcos Organics (Geel, Belgium), and glycine from Serva Electrophoresis GmbH (Heidelberg, Germany). Tauroursodeoxycholic acid (TUDCA) was from Calbiochem (Merck Millipore, Billerica, MA, USA), 2-acetamido-1,2-dideoxynojirimycin (2AcDNJ) from Bio-connect (Life Sciences, Huissen, The Netherlands), and suberanilohydroxamic acid (SAHA or Vorinostat) from Cayman Chemical Company (Ann Arbor, MI, USA).

The compounds used in our screen were first dissolved in milliQ (Synergy Water Purification System, Merck Millipore, Billerica, MA, USA), sterilized using a 0.45 μ m syringe filter (Merck Millipore, Billerica, MA, USA) and diluted in culture medium to the required concentration. Except for UDCA, ambroxol, bortezomib, TSA, and SAHA, for which stock solutions were prepared in DMSO and subsequently diluted in culture medium (final DMSO concentration 1.0%).

The Prestwick Chemical Library (Prestwick Chemical, Illkirch, France) consisted of 1,280 compounds (2 mM stock solutions in DMSO), which were diluted in culture medium to a final concentration of 10 μ M (final DMSO concentration 0.5%).

Western Blot Analysis

Cell pellets were dissolved in milliQ supplemented with cOmplete™ protease inhibitor cocktail (Roche, Mannheim, Germany) and disrupted by sonification using a Vibra Cell sonicator (Sonics & Materials Inc., Newtown, CT, USA). Protein concentration was measured in whole cell lysates as

described by Lowry et al. (1951). For Western blot analysis of NAGLU, 50 μ g of protein was loaded onto a NuPAGE Novex 4–12% Bis-Tris pre-cast polyacrylamide gel (Invitrogen, Carlsbad, CA, USA) that after electrophoresis was transferred onto an Amersham Protran Nitrocellulose Blotting Membrane by semidry blotting (GE Healthcare Life Sciences, Little Chalfont, UK). Membranes were blocked in 30 g/L bovine serum albumin (Sigma-Aldrich, St. Louis, MO, USA) in 0.1% Tween-20 in PBS (TPBS). Antibodies used were: rabbit anti-NAGLU antibody 1:800 (ab169874; Abcam, Cambridge, UK), mouse anti- β -actin antibody 1:10,000 (Sigma-Aldrich, St. Louis, MO, USA), goat anti-rabbit and donkey anti-mouse antibody 1:10,000 (IRDye 800CW and IRDye 680RD, respectively; LI-COR Biosciences, Lincoln, NE, USA). Primary antibodies were dissolved in TPBS and secondary antibodies in TPBS with Odyssey® blocking buffer and SDS 0.01%. Between antibody incubations membranes were washed five times with TPBS. Blots were analyzed using the Odyssey® CLx Infrared Imaging System (LI-COR Biosciences, NE, USA).

Statistical Analysis

Data analyses were performed using SPSS software for Windows (version 23.0, SPSS Inc., Chicago, IL, USA). A *p*-value of <0.05 was considered statistically significant.

Results

Effects of Culturing at 30°C on Mutant NAGLU

Previously it has been shown that residual activity of NAGLU in fibroblasts of MPSIIIB patients with an SP phenotype can be increased by culturing at 30°C (Meijer et al. 2016). To further investigate the increase in activity of mutant NAGLU at low culture temperature, NAGLU protein and activity levels were determined in control and MPSIIIB fibroblast cell lines after culturing at 37 and 30°C (Fig. 1a). Western blot analysis of fibroblasts from a healthy control cultured at 37°C showed that NAGLU consists of two forms: a precursor form with an apparent molecular weight of 85 kD and a mature form with an apparent molecular weight of 82 kD. In the SP p.S612G MPSIIIB cell line, only the precursor form was detected after culturing at 37°C, whereas after culturing at 30°C both NAGLU forms could be observed. This corresponded with an increase in NAGLU activity from 0.41 nmol mg⁻¹ h⁻¹ after culturing at 37°C up to 4.06 nmol mg⁻¹ h⁻¹ after culturing at 30°C (Fig. 1b; NAGLU activity in control fibroblasts cultured at 37°C: 19.71 nmol mg⁻¹ h⁻¹). In fibroblasts of the RP p.R297* MPSIIIB patient, no NAGLU

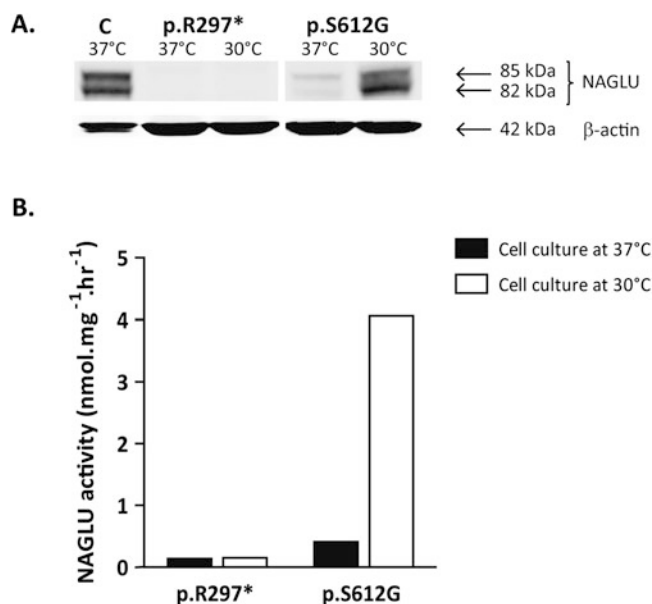


Fig. 1 (a) Western blot analysis of NAGLU protein levels and (b) corresponding activity levels ($\text{nmol mg}^{-1} \text{h}^{-1}$) after culturing MPSIIIB patient and control fibroblasts at 37 and 30°C for 1 week.

NAGLU activity was measured as was described previously (Meijer et al. 2016). NAGLU activity in control fibroblasts cultured at 37°C was $19.71 \text{ nmol mg}^{-1} \text{h}^{-1}$

protein was present under either of these conditions (measured NAGLU activities: 0.14 and $0.15 \text{ nmol mg}^{-1} \text{h}^{-1}$ after culturing at 37°C and 30°C, respectively).

Optimization and Validation of the 96-Well NAGLU Assay

Prior to the compound screen, a method suitable for high-throughput applications was developed based on the method described by Mauri et al. (2013) and optimized for incubation time, 4MU- α -GlcNAc substrate concentration and cell density (Supplementary figure 1). Based on these results we decided to use 10,000 cells/well and to measure NAGLU activity after 5 days of culturing using 1 mg/mL 4MU- α -GlcNAc substrate incubated at 37°C for 24 h. Since at present no compound is known that can enhance residual NAGLU activity in MPSIIIB fibroblasts, fibroblasts of a healthy subject were used as a positive control and plated at a density of 2,500 cells/well in each plate. As chaperones only act on missense variants, p.R297* MPS IIIB fibroblasts plated at a density of 10,000 cells/well were included as a negative control. Since this cell line contains a mutation resulting in a premature stop, no protein will be synthesized and no activity was expected to be measured.

Using these conditions, the Z-factor of the assay was determined, which is considered a reliable measure for evaluation and validation of high-throughput screens (Zhang et al. 1999). A calculated Z-factor of 0.69 classified this assay as “excellent.”

Effect of Chemical Chaperones

Chemical chaperones are generally divided into two subgroups: the osmolytes and hydrophobic compounds (Cortez and Sim 2014). Several classes of osmolytes were studied: free amino acids and amino acid derivatives (β -alanine, glycine, taurine, D-arginine, L-homoarginine hydrochloride, L-arginine monohydrochloride), carbohydrates (trehalose and saccharose), polyols (glycerol), methylamines (betaine and TMAO), and organosulfur compounds (DMSO). In addition the effect of the hydrophobic chaperones 4-PBA and the bile acids UDCA and TUDCA was assessed.

None of the 15 chemical chaperones tested, enhanced NAGLU activity in MPSIIIB fibroblasts after 5 days incubation at different concentrations (Fig. 2a).

Effect of Pharmacological Chaperones, Previously Investigated in LSDs

Pharmacological chaperones previously studied for potential effects in LSDs were assessed and included ambroxol (Maegawa et al. 2009), the proteasome inhibitor bortezomib (Shimada et al. 2011; Macías-Vidal et al. 2014) and the HDAC inhibitors TSA and SAHA (Pipalia et al. 2011). None of these compounds enhanced residual NAGLU activity in p.S612G MPSIIIB fibroblasts (Fig. 2b).

The (*N*-acetyl)glucosamine inhibitors D-glucosamine, *N*-acetylglucosamine, and 2AcDNJ, which were previously

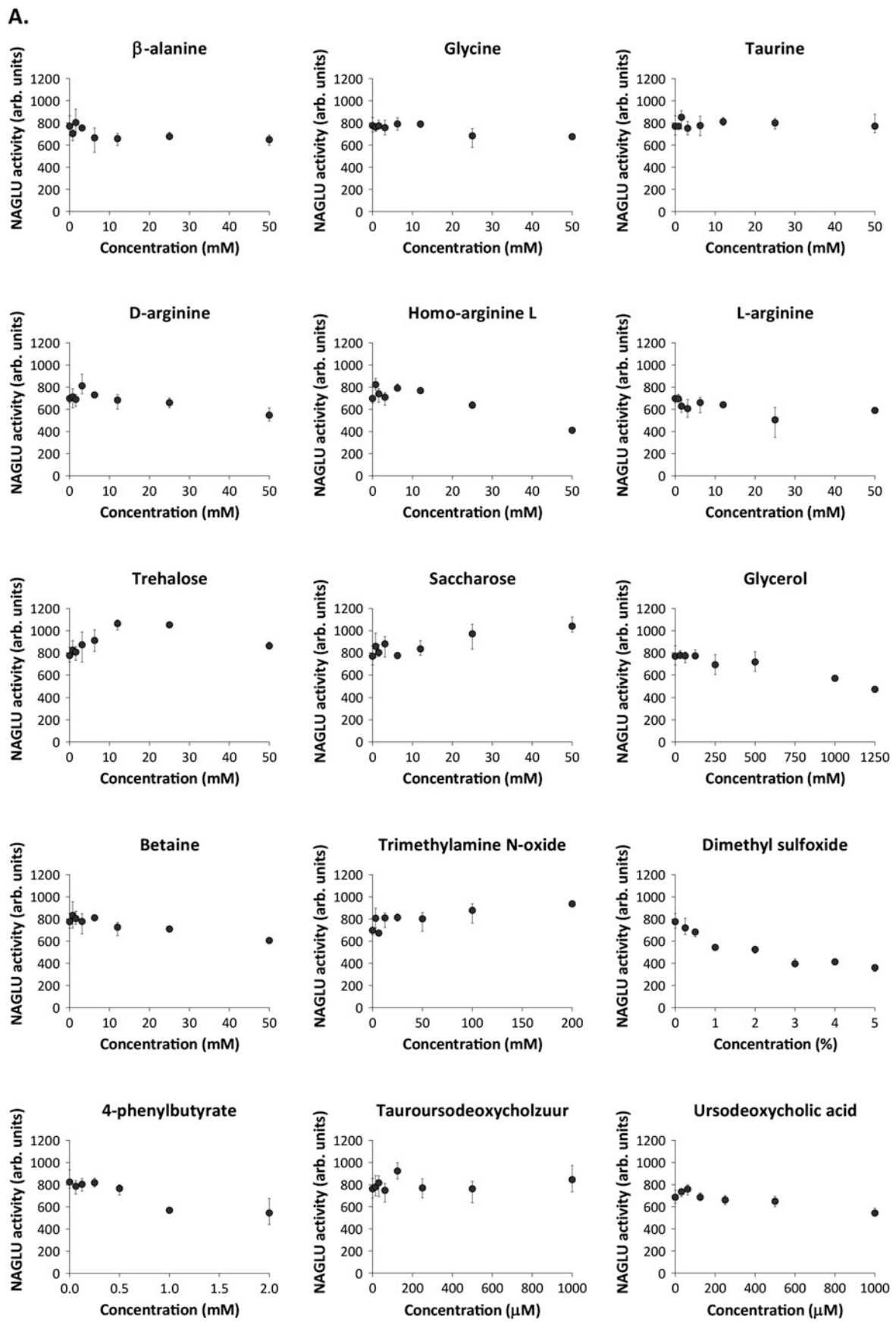


Fig. 2 (continued)

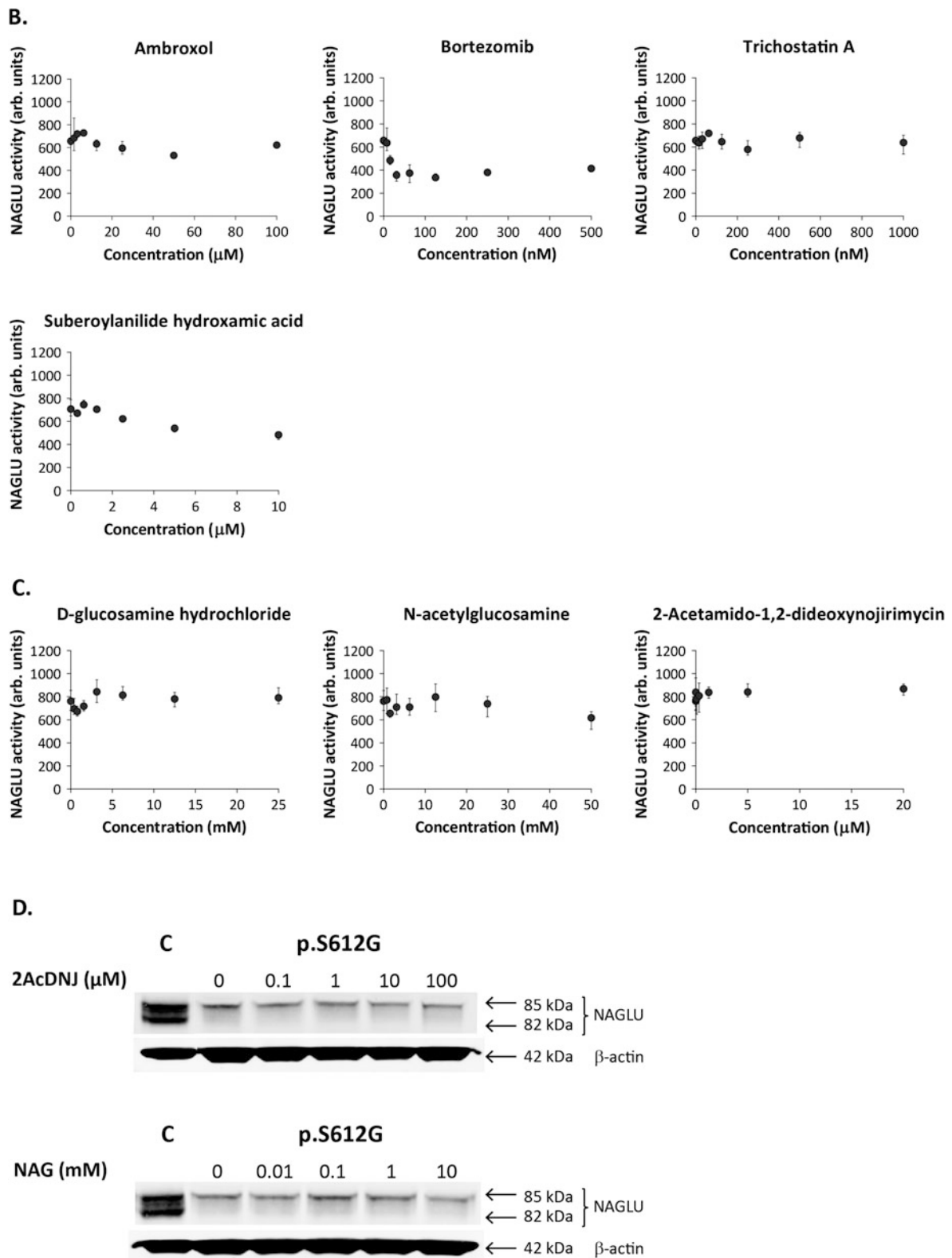


Fig. 2 (a) Effect of treatment with different classes of chemical chaperones on residual NAGLU activity in p.S612G MPSIII B fibroblasts. (b) Effect of treatment with pharmacological chaperones used in other protein folding diseases including LDSs, on residual NAGLU activity in p.S612G MPSIII B fibroblasts. (c) Effect of treatment with pharmacological chaperones used in MPS III on

residual NAGLU activity in p.S612G MPSIII B fibroblasts. NAGLU activity levels are shown in fluorescence (arb. units). All compound concentrations were tested in triplicate. Mean \pm SD is given. (d) Western blot analysis of the effect of treatment with *N*-acetylglucosamine (NAG) and 2AcDNJ for 5 days on NAGLU protein levels in p.S612G MPSIII B fibroblasts

shown to affect NAGLU and HGSNAT in MPSIIIB and MPS IIIC respectively, were also investigated (Zhao and Neufeld 2000; Ficko-Blean et al. 2008; Feldhammer et al. 2009; Matos et al. 2014). As is shown in Fig. 2c, treatment with these compounds did not lead to significant enhancement of NAGLU activity levels.

To investigate whether any of these compounds did have an effect on protein levels, which, due to a too strong inhibitory effect, may not have resulted in detectable changes in enzyme activity, the effect of *N*-acetylglucosamine and 2AcDNJ on NAGLU protein was assessed on Western blot (Fig. 2d). Treatment with neither of these compounds led to higher expression of the precursor of NAGLU or the formation of the mature form of the enzyme.

Prestwick Chemical Library

So far none of the compounds tested showed any effect on residual activity of mutant NAGLU. Therefore the Prestwick Chemical Library was tested consisting of 1,280 approved drugs (Fig. 3a). All compounds with a fluorescent signal of 500 arb. units or above the mean background were selected for further analyses. These included the antihypertensive drugs benzamil hydrochloride (PCL-657) and doxazosin mesylate (PCL-858), the anti-osteoporetic drug ibandronate sodium (PCL-1285), the xanthine oxidase inhibitor used for the treatment of gout, allopurinol (PCL-1213), and the antiseptic drug aminacrine (PCL-1717). To validate the results of the screen, MPSIIIB p.S612G fibroblasts were incubated for 5 days with the selected compounds at increasing concentrations. Both ibandronate sodium and allopurinol did not show any significant effect on NAGLU activity (Fig. 3b). For aminacrine, benzamil hydrochloride, and doxazosin mesylate a dose dependent increase in fluorescent signal was observed. However, the same increase in fluorescence was obtained when the assay was repeated in the absence of the 4MU- α -GlcNAc substrate, indicating that there is no actual increase in residual NAGLU activity upon treatment with these compounds.

Discussion

We assessed the effect of 1,302 different molecules on residual enzyme activity in a MPSIIIB patient fibroblast cell line which responded with a significant increase in NAGLU activity when cultured at 30°C instead of 37°C (Meijer et al. 2016). As enzyme activity is related to the efficiency of protein folding, culturing at 30°C may improve folding of the mutant NAGLU enzyme (Fan 2003; Gootjes et al. 2004). Unfortunately, none of the molecules tested in our assay,

including the 1,280 compounds from the Prestwick Chemical Library, were effective.

The observed lack of effect may well be understood if protein misfolding is not, or only to a limited extent, involved in the SP MPSIIIB phenotype and if the observed increase in enzyme activity in fibroblasts cultured at 30°C is due to other mechanisms. Indeed, our observation on Western blot that at 37°C culture conditions only the 85 kDa precursor form of NAGLU is detected while at 30°C also the mature 82 kDa NAGLU protein is observed rather suggests differences in protein synthesis and processing.

The majority of chemical chaperones studied here have remarkable general mechanisms of action and were shown to influence enzymatic activity in other protein folding diseases including LSDs (Maegawa et al. 2009; Pipalia et al. 2011; Shimada et al. 2011; Cortez and Sim 2014; Macías-Vidal et al. 2014). We consider that, if protein misfolding is indeed involved in the MPSIIIB SP phenotype, some effect of these compounds would have been observed. Our observation that more NAGLU specific compounds such as the *N*-acetylglucosaminidase inhibitors *N*-acetylglucosamine and 2AcDNJ also lacked effect on NAGLU protein and activity levels further supports the hypothesis that protein misfolding does not play a major role in MPSIIIB. In previous studies the binding capacity of 2AcDNJ has always been assessed using purified NAGLU (Zhao and Neufeld 2000; Ficko-Blean et al. 2008). As NAGLU is synthesized in the rough endoplasmic reticulum (ER), it is possible that these compounds do have the capacity to bind and stabilize mutant NAGLU, but cannot enter the ER in a cell culture model as used here. This may also have blocked potential effects of other compounds tested in this study, although it is unlikely that compounds which do not reach the target protein in vitro would have therapeutic properties in vivo. Thus, despite the promising effects of chaperones in other LSDs such as Fabry disease, this approach may not serve all LSDs as was shown here for MPS IIIB (Hollak and Wijburg 2014; Germain et al. 2016; Hughes et al. 2017).

A limitation of this study is that compounds were tested in one MPSIIIB cell line. Although this cell line was selected because its mutation conveys an SP phenotype and enzyme activity responded favorably to culturing at 30°C, we cannot exclude that this mutation is insensitive to the here tested compounds and that they might have had a positive effect on other mutations. However, allelic heterogeneity in MPSIIIB is large, and it would not be feasible to test all reported mutations (Valstar et al. 2010). A drawback of high-throughput screens in general is that compound libraries are often tested in a limited number of concentrations, so that an effect of any of the compounds at a different concentration cannot be ruled out.

Thus, despite a reliable and robust assay, this high-throughput screen failed to identify compounds that could

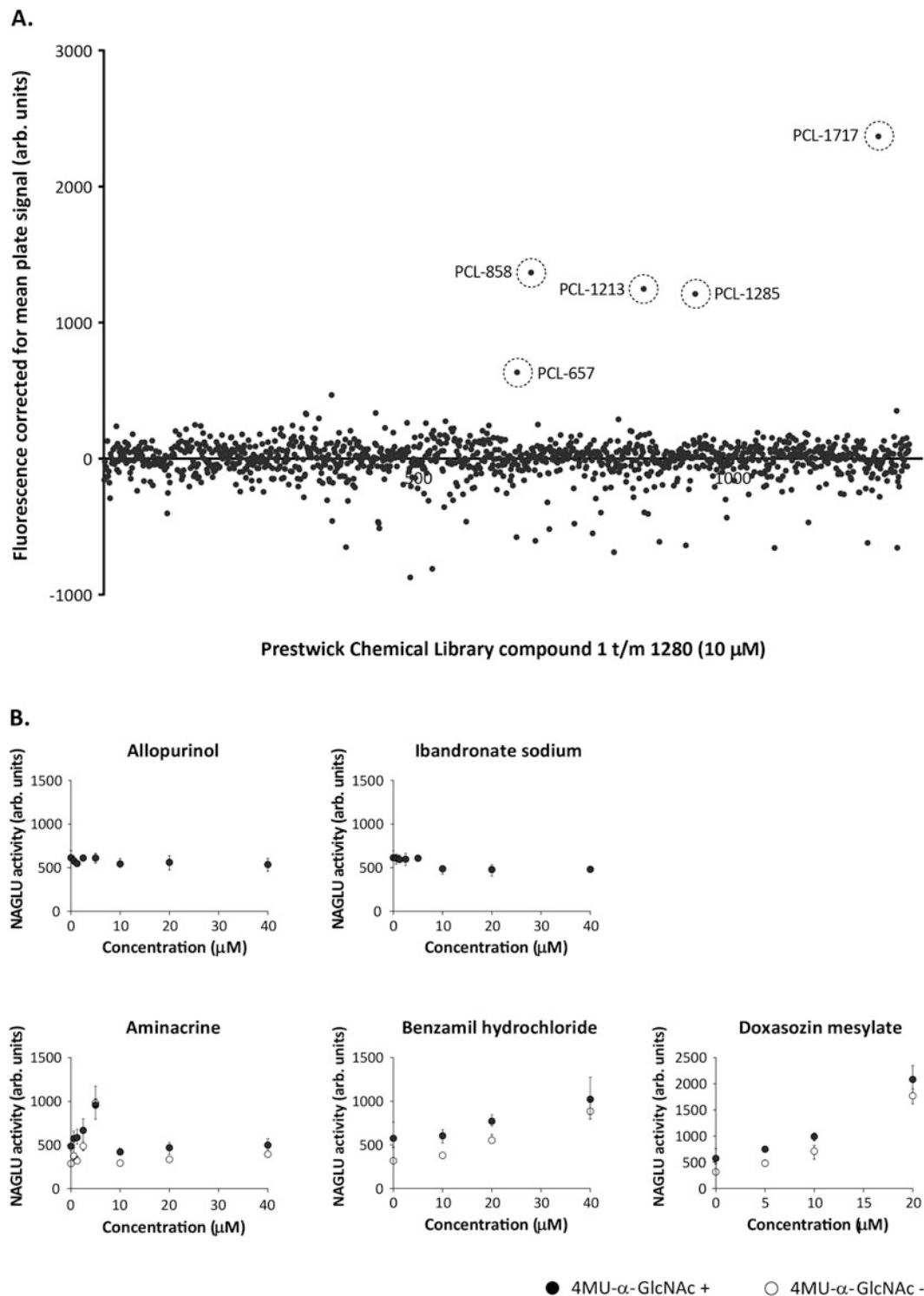


Fig. 3 (a) Effect of treatment with compounds from the Prestwick Chemical Library (10 μ M) on NAGLU activity in p.S612G MPSIIIB fibroblasts. NAGLU activity levels are shown in fluorescence (arb. units) and were corrected for the mean plate signal. (b) Validation of the compounds identified in the high-throughput screen of the

Prestwick Chemical Library in p.S612G MPSIIIB fibroblasts. NAGLU activity levels are shown in fluorescence (arb. units) after incubation with or without 4MU- α -GlcNAc substrate. All compound concentrations were tested in triplicate. Mean \pm SD is given

enhance residual activity of mutant NAGLU in fibroblasts of an SP MPSIIIB patient homozygous for a temperature

sensitive mutation. We conclude that to therapeutically simulate the positive effect of lower temperatures on

residual NAGLU activity, first more insight is needed into the mechanisms underlying this temperature dependent increase in enzyme activity.

Acknowledgements The authors would like to thank Dr. S. F. van de Graaf of the Tytgat Institute for Liver and Intestinal Research/ Department of Gastroenterology & Hepatology at the Academic Medical Center in Amsterdam, for being so kind to provide the Prestwick Chemical Library to us. This study was funded by grants from the private foundations “Stichting Stofwisselkracht,” “Zabawas,” “Zeldzame Ziekten Fonds,” and “Kinderen en Kansen,” the Netherlands.

Take Home Message

High-throughput screen fails to identify compounds that enhance residual enzyme activity of mutant *N*-acetyl- α -glucosaminidase in mucopolysaccharidosis type IIIB.

Author Contributions

O. L. M. Meijer: Designed and conducted the study, was responsible for data analysis and interpretation and for writing of the article
 P. van den Biggelaar: Assisted in the conduction of the study
 R. Ofman: Assisted in the design and conduction of the study
 F. A. Wijburg: Designed and supervised the study, revised the manuscript
 N. van Vlies: Designed and supervised the study, revised the manuscript

Guarantor for the Article

F. A. Wijburg, MD PhD, Department of Pediatric Metabolic Diseases, Emma Children’s Hospital and Amsterdam Lysosome Center “Sphinx,” Academic Medical Center, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands, f.a.wijburg@amc.uva.nl.

Authors Conflict of Interest

O. L. M. Meijer, P. van den Biggelaar, R. Ofman, F. A. Wijburg, and N. van Vlies declare that they have no conflict of interest.

Details of Funding

This study was funded by grants from the private foundations “Stichting Stofwisselkracht,” “Zabawas,” “Zeldzame Ziekten Fonds,” and “Kinderen en Kansen,” the Netherlands.

Details of Ethics Approval

No ethics approval was required for this study. This article does not contain any studies with human or animal subjects performed by any of the authors.

Informed Consent

Informed consent for the use of patient fibroblasts was obtained from parents or legal representatives for all patients.

Animal Rights

This article does not contain any studies with animal subjects performed by any of the authors.

References

- Cortez L, Sim V (2014) The therapeutic potential of chemical chaperones in protein folding diseases. *Prion* 8:197–202
- Engin F, Hotamisligil GS (2010) Restoring endoplasmic reticulum function by chemical chaperones: an emerging therapeutic approach for metabolic diseases. *Diabetes Obes Metab* 12:108–115
- Fan JQ (2003) A contradictory treatment for lysosomal storage disorders: inhibitors enhance mutant enzyme activity. *Trends Pharmacol Sci* 24:355–360
- Feldhammer M, Durand S, Pshezhetsky AV (2009) Protein misfolding as an underlying molecular defect in mucopolysaccharidosis III type C. *PLoS One* 4:e7434
- Ficko-Blean E, Stubbs KA, Nemirovsky O et al (2008) Structural and mechanistic insight into the basis of mucopolysaccharidosis IIIB. *Proc Natl Acad Sci U S A* 105:6560–6565
- Germain DP, Hughes DA, Nicholls K et al (2016) Treatment of Fabry’s disease with the pharmacologic chaperone migalastat. *N Engl J Med* 375:545–555
- Gootjes J, Schmohl F, Mooijer PA et al (2004) Identification of the molecular defect in patients with peroxisomal mosaicism using a novel method involving culturing of cells at 40°C: implications for other inborn errors of metabolism. *Hum Mutat* 24:130–139
- Hartl FU, Bracher A, Hayer-Hartl M (2011) Molecular chaperones in protein folding and proteostasis. *Nature* 475:324–332
- Hollak CE, Wijburg FA (2014) Treatment of lysosomal storage disorders: successes and challenges. *J Inher Metab Dis* 37:587–598
- Hughes DA, Nicholls K, Shankar SP et al (2017) Oral pharmacological chaperone migalastat compared with enzyme replacement therapy in Fabry disease: 18-month results from the randomised phase III ATTRACT study. *J Med Genet* 54:288–296
- Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. *J Biol Chem* 193:265–275
- Macias-Vidal J, Girós M, Guerrero M et al (2014) The proteasome inhibitor bortezomib reduced cholesterol accumulation in fibroblasts from Niemann-Pick type C patients carrying missense mutations. *FEBS J* 281:4450–4466
- Maegawa GH, Tropak MB, Buttner JD et al (2009) Identification and characterization of ambroxol as an enzyme enhancement agent for Gaucher disease. *J Biol Chem* 284:23502–23516

- Matos L, Canals I, Dridi L et al (2014) Therapeutic strategies based on modified U1 snRNAs and chaperones for Sanfilippo C splicing mutations. *Orphanet J Rare Dis* 9:180
- Mauri V, Lotfi P, Segatori L, Sardiello M (2013) A rapid and sensitive method for measuring N-acetylglucosaminidase activity in cultured cells. *PLoS One* 8:1–9
- Meijer OL, Welling L, Valstar MJ et al (2016) Residual N-acetyl- α -glucosaminidase activity in fibroblasts correlates with disease severity in patients with mucopolysaccharidosis type IIIB. *J Inher Metab Dis* 39:437–445
- Moog U, van Mierlo I, van Schrojenstein Lantman-de Valk HM et al (2007) Is Sanfilippo type B in your mind when you see adults with mental retardation and behavioral problems? *Am J Med Genet C Semin Med Genet* 145C:293–301
- Muenzer J (2011) Overview of the mucopolysaccharidoses. *Rheumatology* 50(suppl 5):v4–v12
- Parenti G (2009) Treating lysosomal storage diseases with pharmacological chaperones: from concept to clinics. *EMBO Mol Med* 1:268–279
- Parenti G, Andria G, Valenzano KJ (2015) Pharmacological chaperone therapy: preclinical development, clinical translation, and prospects for the treatment of lysosomal storage disorders. *Mol Ther* 23:1138–1148
- Pipalia NH, Cosner CC, Huang A et al (2011) Histone deacetylase inhibitor treatment dramatically reduces cholesterol accumulation in Niemann-Pick type C1 mutant human fibroblasts. *Proc Natl Acad Sci U S A* 108:5620–5625
- Shimada Y, Nishida H, Nishiyama Y et al (2011) Proteasome inhibitors improve the function of mutant lysosomal α -glucosidase in fibroblasts from Pompe disease patient carrying c.546G>T mutation. *Biochem Biophys Res Commun* 415:274–278
- Valstar MJ, Bruggenwirth HT, Olmer R et al (2010) Mucopolysaccharidosis type IIIB may predominantly present with an attenuated clinical phenotype. *J Inher Metab Dis* 33:759–767
- Zhang JH, Chung TD, Oldenburg KR (1999) A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J Biomol Screen* 4:67–73
- Zhao KW, Neufeld EF (2000) Purification and characterization of recombinant human α -N-acetylglucosaminidase secreted by Chinese hamster ovary cells. *Protein Expr Purif* 19:202–211