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

**Mucopolysaccharidosis type II (MPS II, Hunter disease) is an X chromosome-linked inherited metabolic disease caused by mutations resulting in deficiency of activity of iduronate-2-sulfatase (IDS) and accumulation of undegraded glycosaminoglycans (GAGs), heparan sulfate, and dermatan sulfate. Previous experiments with cell cultures and studies on animal model of MPS II suggested that gene expression-targeted isoflavone therapy (GET IT), based on genistein-mediated reduction of efficiency of GAG synthesis, might be a suitable therapy for this disease. In this report, we demonstrate efficacy of GET IT in connective tissue elasticity, particularly in improving the range of joint motion in seven patients with MPS II after 26 weeks of treatment with an isoflavone extract at the dose corresponding to 5 mg/kg/day of genistein.** © 2011 Wiley-Liss, Inc.

**Key words:** gene expression-targeted isoflavone therapy; genistein; Hunter disease; mucopolysaccharidoses; joint motion; substrate reduction therapy

**How to Cite this Article:**
Marucha J, Tylki-Szymańska A, Jakóbkiewicz-Banecka J, Piotrowska E, Kloska A, Czartoryska B, Węgrzyn G. 2011. Improvement in the range of joint motion in seven patients with mucopolysaccharidosis type II during experimental gene expression-targeted isoflavone therapy (GET IT).
Am J Med Genet Part A 9999:1–6.

## INTRODUCTION

Mucopolysaccharidosis type II (also called MPS II or Hunter syndrome, OMIM +309900) is an X chromosome-linked disorder caused by mutations resulting in a deficiency of the activity of the lysosomal enzyme, iduronate-2-sulfatase (IDS) [Neufeld and Muenzer, 2001]. This enzyme is involved in degradation of dermatan sulfate (DS) and heparan sulfate (HS). Impaired degradation of these glycosaminoglycans (GAGs) results in accumulation of undegraded DS and HS in various tissues and organs, contributing to the signs and symptoms of the disease. These are caused by progressive damage of the affected tissues, including the heart, respiratory system, bones, joints, and central nervous system (behavioral problems and mental retardation occur in most patients, although some patients have normal cognitive development) [Neufeld and Muenzer, 2001].

Recently, enzyme replacement therapy (ERT) has been developed and is available for treatment of patients with three types of MPS: MPS I, MPS II, and MPS VI [Rohrbach and Clarke, 2007; Lim-Melia and Kronn, 2009]. This therapy is effective in ameliorating some symptoms; however, intravenous infusion of human recombinant enzyme is not effective in treatment of central nervous

Abbreviations: DS, dermatan sulfate; ERT, enzyme replacement therapy; HS, heparan sulfate; GAG, glycosaminoglycan; GET IT, gene expression-targeted isoflavone therapy; IDS, iduronate-2-sulfatase; IQ, intelligence quotient; MPS, mucopolysaccharidosis; ROM, range of motion; SRT, substrate reduction therapy.

Grant sponsor: Ministry of Science and Higher Education of Poland; Grant numbers: N302 046 32/3603, 3631/B/P01/2007/33; Grant sponsor: European Regional Development Fund; Grant number: TEAM/2008-2/7.

*Correspondence to:
Grzegorz Węgrzyn, Department of Molecular Biology, University of Gdańsk, Kadki 24, 80-822 Gdańsk, Poland.
E-mail: wegrzyn@biotech.univ.gda.pl

Published online 00 Month 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.34146

system as the enzyme cannot cross the blood–brain barrier. Moreover, other problems exist with this therapy. For example, there is low efficiency of penetration of the recombinant enzyme to bones and joints [Rohrbach and Clarke, 2007; Lim-Melia and Kronn, 2009]. As a consequence, no statistically significant improvement has been noted in growth efficiency [Tylki-Szymanska et al., 2010b] and joint motion [Cox-Brinkman et al., 2007] among MPS I patients treated with ERT, although patients with normal or near normal mental development who underwent active physical rehabilitation showed improvement in active movement [Tylki-Szymanska et al., 2010a].

Gene expression-targeted isoflavone therapy (GET IT) is another experimental option for treatment of MPS patients [Piotrowska et al., 2006, 2008, 2011]. This therapy is based on the use of genistein, an isoflavone (4′,5,7-trihydroxyisoflavone or 5,7-dihydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one), which indirectly impairs the efficiency of GAG synthesis through inhibition of phosphorylation of epidermal growth factor receptor [Jakobkiewicz-Banecka et al., 2009]. In vitro studies suggested that genistein slows synthesis of at least some GAGs, particularly DS and HS [Piotrowska et al., 2006]. Slow GAG synthesis appears to prevent further accumulation of these substances in patient's cells, and residual activity of the deficient enzyme may cause a reduction in the level of already accumulated compounds and achievement of a new balance between GAG synthesis and degradation [Wegrzyn et al., 2010]. As such, GET IT is a specific case of substrate reduction therapy (SRT), which groups the treatment procedures leading to impairment of production of compounds (substrates) that cannot be efficiently degraded.

Until now, GET IT was investigated mostly as an experimental therapy for MPS type III (Sanfilippo disease), mainly because no other treatment is currently available for this MPS disorder [Wegrzyn et al., 2010]. However, patients with other MPS disorders, even those treated with ERT, might potentially benefit from GET IT therapy. This concerns mostly patients with either neurological symptoms or bone and joint dysfunction, due to ineffective delivery of intravenously administered recombinant enzymes to these organs. Although positive effects of GET IT on central nervous system functions were demonstrated during a pilot clinical study with MPS III patients [Piotrowska et al., 2008, 2011], no results on efficacy of this therapy in improving joint functions in patients have been reported. On the other hand, positive results of GET IT therapy of mice with MPS IIIB [Malinowska et al., 2009] and MPS II [Friso et al., 2010] encouraged us to perform experimental therapy of patients with MPS II, in whom joint problems were severe.

## PATIENTS AND METHODS

### Patients

Seven patients, in whom MPS II was diagnosed by demonstration of increased urinary GAG levels and deficient activity of IDS in leukocytes or plasma [Pennock, 1976; Voznyi et al., 2001], were investigated. These male patients were 8–25 years old at the time of the beginning of treatment. Very low residual activities of IDS (in the range of 0.6–28 pmol/ml/hr; normal mean value: $500 \pm 200$ pmol/ml/hr) were identified in all patients (Table I). The same mutation, c.1034G > T, was detected in the *IDS* gene of Patients no. 3, 4, and 5, who are relatives, and other mutations were identified in Patients 1, 2, 6, and 7. A summary of characteristics of patients is provided in Table I. All of them were of normal or near-normal intelligence; thus they could cooperate with investigators (by responding to commands) during examination. The intellectually and mentally well-developed older children (age 8–10 years) and adult patients, rather than cognitively and mentally handicapped young children, were enrolled in this study due to: (i) better communication and collaboration with patients during various tests, (ii) severe symptoms occurring in joints of such patients (common features of MPS II are joint stiffness and restriction of the range of motion, ROM, which increase with age), and (iii) a lack of ERT as an option for these patients at the time of the start of this study.

### Treatment

The experimental therapy was performed according to the approval by the Independent Bioethics Committee of the Medical University of Gdańsk, Poland. Patients involved in this study have signed an

TABLE I. Patients' Characteristics

| Patient no. | Age at diagnosis (years) | Age at baseline (years) | IQ at baseline | Hepatosplenomegaly[a] | Urinary GAG excretion at diagnosis (mg/g of creatinine) | Residual IDS activity[b] | Mutation in the *IDS* gene |
|---|---|---|---|---|---|---|---|
| 1 | 4 | 8 | 70 | ++ | 596 | 1 (*) | p.R493P |
| 2 | 8.5 | 10 | 93 | + | 705 | 0.8 (*) | c.181T > C |
| 3 | 3 | 22 | 66 | ++ | 738 | 0.6 (**) | c.1034G > T |
| 4 | 1.5 | 24 | 72 | – | 585 | 0.8 (**) | c.1034G > T |
| 5 | 5 | 25 | 99 | ++ | 577 | 1.0 (**) | c.1034G > T |
| 6 | 6.5 | 10 | 85 | + | 307 | 9.2 (*) | c.1048A > C |
| 7 | 9 | 9 | 95 | – | 600 | 28 (*) | c.1030G > A |

[a]Hepatosplenomegaly was assessed as: none (–), increased by less than 2 cm in each dimension (+), increased by more than 2 cm in one or more dimensions (++) as measured by ultrasonography.
[b]Residual IDS activity was measured in either serum (*, in pmol/ml/hr) or in cultures skin fibroblasts (**, nmol/mg of protein/24 h).

informed consent form. All patients received a genistin-rich soy isoflavone extract (SE-2000, also called Soyfem, provided by Biofarm Company, Poznań, Poland) at the dose corresponding to 5 mg of genistein and genistin (a glycoside) per 1 kg of body mass daily for 6 months. This dose was chosen on the basis of previously described pilot clinical studies with MPS III patients [Piotrowska et al., 2008].

## Determination of Joint Range of Motion

Active and passive ROM of joints of upper and lower extremities were measured using a double-armed goniometer at the baseline and after 26 weeks of the treatment, as described previously [Gerhardt and Rondinelli, 2001; Tylki-Szymanska et al., 2010a,b]. Results were recorded in the SFTR system (International Method of Measuring and Recording Joint Motion). Since the range of active shoulder flexion may be influenced by spinal retroflexion, in this test, the ROM was measured with the thorax, rather than the vertical plane, as a reference.

## Statistical Analysis

Due to small number of patients, descriptive statistics were used, and the results are presented as median and range. The choice of such a method was based on the power and sample size calculation, performed using power test Version 2.1.31 (PS). Values measured before and after treatment were compared using the paired *t*-test (one-tailed) with statistical significance level of $P < 0.05$. Calculations were performed using Statistica 8.0 software (StatSoft).

## RESULTS AND DISCUSSION

We found that 6 months of oral administration of genistein-rich soy isoflavone extract resulted in improvement in active and passive shoulder ROM in all patients (Fig. 1, Table II). The range of such an improvement was from 10° to 30° and was statistically significant in the group of all patients (Table II). Less pronounced, but still clearly observable improvement in motion of other joints (elbow and wrist) in the upper extremities were also observed in some patients (Table II), though these changes were not statistically significant in the group of all patients, apparently due to a relatively high variability between the patients. Some improvement in four out of seven patients was observed in motion of lower extremity joints (Table III). However, in the tested patients, restrictions in ROM of lower extremities were generally milder than those of the upper extremities. The values for lower extremities measured in patients before treatment were often equal to or close to normal values, and the changes were not statistically significant in the group of all patients (Table III). One may speculate that this difference between lower and upper extremities arises due to a kind of "natural rehabilitation" of the lower extremities, as walking is a natural movement and natural kind of activity of patients, which keeps motion of joints at relatively high level, despite the level of GAG accumulation in the joints.

Although all patients have increased urinary GAG levels, no correlation could be detected between the amount of excreted GAGs and joint mobility or intelligence (Table I and calculations not shown). Moreover, urinary GAG levels in particular patients did not correlate with effects of GET IT on joint motion.

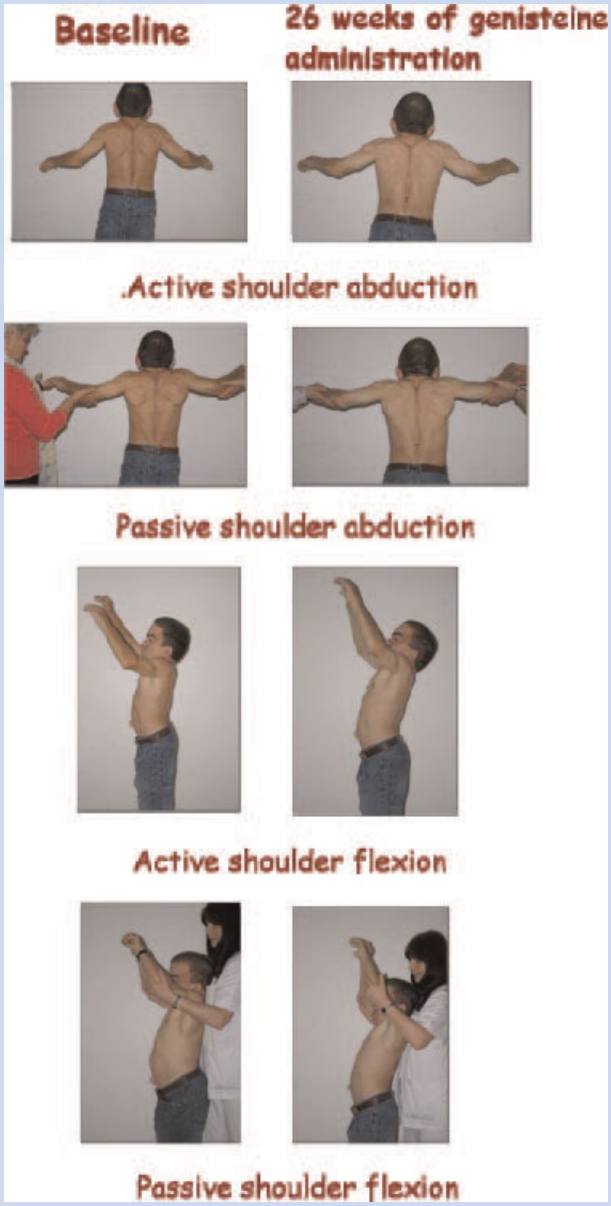


**FIG. 1. Active and passive shoulder flexion and abduction in Patient no. 4 at baseline and after 26 weeks of using regularly 5 mg of genistein/kg daily.**

Adverse effects were monitored throughout the period of treatment, and none were observed during treatment. The increased ranges of motion in joints indicate that administration of genistein, at the dose of 5 mg/kg/day, may be considered as a promising method to improve joint ROM, and thus, improved performance in daily living of MPS II patients. These results represent the first evidence that GET IT may be an efficient treatment of some symptoms of MPS II in humans. On the other hand, since this was an open-label study and patients collaborated with examining persons during the tests, a placebo effect cannot be excluded.

TABLE II. Active and Passive Joint ROM in the Upper Extremities at Baseline and After GET IT

| Patient no., norm value or parameter | Shoulder flexion (°) | Shoulder abduction (°) | Elbow extension (°) | Elbow flexion (°) | Wrist extension (°) | Wrist flexion (°) |
|---|---|---|---|---|---|---|
| Active joint ROM | | | | | | |
| Norm | 175 | 175 | 0 | 150 | 70 | 80 |
| Patient 1 | 80/100* | 80/100* | 30/40 | 150/150 | 20/20 | 30/30 |
| Patient 2 | 110/110* | 80/100* | 40/30* | 150/150 | 20/10 | 70/0 |
| Patient 3 | 100/110* | 60/90* | 70/50* | 130/130 | 0/5* | 20/40* |
| Patient 4 | 90/110* | 70/100* | 60/60 | 120/120 | 0/10* | 5/20* |
| Patient 5 | 100/120* | 70/90* | 50/30* | 150/130 | 10/10 | 30/50* |
| Patient 6 | 100/120* | 90/110* | 40/35* | 150/150 | 20/20 | 25/25 |
| Patient 7 | 100/120* | 90/90 | 25/25 | 140/140 | 0/0 | 10/20* |
| Mean | 97.1/112.9 | 77.1/97.1 | 45.0/38.6 | 141.4/138.6 | 10.0/10.7 | 27.1/26.4 |
| SD | 9.5/7.6 | 11.1/7.6 | 16.1/12.5 | 12.1/12.1 | 10.0/7.3 | 21.2/16.0 |
| *t*-Test *P*-value | 0.001 (s) | 0.001 (s) | 0.088 (n.s.) | 0.178 (n.s.) | 0.383 (n.s.) | 0.477 (n.s.) |
| Passive joint ROM | | | | | | |
| Norm | 180 | 180 | 0 | 155 | 80 | 90 |
| Patient 1 | 120/120 | 120/120 | 30/40 | 150/150 | 20/20 | 30/30 |
| Patient 2 | 130/130 | 90/120 | 40/30* | 150/150 | 20/10 | 70/50 |
| Patient 3 | 120/130* | 80/100* | 70/50* | 130/130 | 0/5* | 20/40* |
| Patient 4 | 120/120 | 80/115* | 60/60 | 120/120 | 0/10* | 5/20* |
| Patient 5 | 110/140* | 90/100* | 50/30* | 150/130 | 10/10 | 30/50* |
| Patient 6 | 120/130* | 100/130* | 40/35* | 150/150 | 20/20 | 25/40* |
| Patient 7 | 120/135* | 100/110* | 25/25 | 150/150 | 10/10 | 20/30* |
| Mean | 120.0/129.3 | 94.3/113.6 | 45.0/38.6 | 142.9/140.0 | 11.4/12.1 | 28.6/37.1 |
| SD | 5.8/7.3 | 14.0/11.1 | 16.1/12.5 | 12.5/12.9 | 9.0/5.7 | 20.1/11.1 |
| *t*-Test *P*-value | 0.033 (s) | 0.004 (s) | 0.088 (n.s.) | 0.178 (n.s.) | 0.383 (n.s.) | 0.083 (n.s.) |

s, Statistically significant; n.s., not significant.
Values before slash indicate results before GET IT, and values after slash those measured after GET IT. Asterisk indicates improvement after GET IT.

Nevertheless, an improvement of passive joint motion in most patients (Tables II and III) suggests that, in this case, a placebo effect is unlikely.

In the two reports on the use of GET IT in treatment of patients with MPS [Piotrowska et al., 2008, 2011], three basic tests were employed to evaluate the efficacy of therapy: (i) urinary GAG level determination, (ii) hair morphology analysis, and (iii) psychological test to assess cognitive functions. However, none of these tests could be employed in this study. Firstly, although urinary GAG level determination appears to be a suitable test in assessment of the effects of treatment of pediatric MPS patients, it has been demonstrated that amounts of GAGs excreted with urine decrease considerably with age [Tomatsu et al., 2005]. Age-related changes in GAGs were reported not only in urine but also in various human tissues and organs [Murata and Horiuchi, 1978; Olczyk, 1993; Carrino et al., 2000]. Thus, in older children and adult MPS patients, urinary GAG levels are relatively low, and do not provide a useful parameter in assessment of efficacy of a treatment. Secondly, changed morphology of hair strands, determined under electron microscope, was reported in various MPS types, including MPS II [Malinowska et al., 2008]. However, such changes are evident in more severe patients rather than in the attenuated cases of MPS II, like the patients in this report. Therefore, the hair morphology test appeared useless in this study. Thirdly, since normal or near-normal intelligence was estimated in patients enrolled in this study, the use of a psychological test to assess treatment efficacy was not considered. Hence, measurement of joint motion appeared to be a suitable assay for estimation of efficiency of GET IT in older children and adults with MPS II.

In the literature published to date, there is a limited number of reports on effects of other therapeutic procedures, particularly ERT, on joints' motion in MPS II patients. Therefore, it is difficult to compare efficiencies of GET IT and ERT in improving these parameters. In clinical trials with ERT for MPS II, the 6 min walking test was used; however, this test can illustrate motion of lower extremities only to some extent as functions of other tissues and organs (e.g., mussels, blood circulation, and others) influence the results of this test considerably [for reviews and comments on ERT for MPS II see Burrow and Leslie, 2008; Wraith et al., 2008]. Nevertheless, Okuyama et al. [2010] tested 10 MPS II patients at ages between 21 and 53 years who received weekly intravenous infusions of 0.5 mg/kg idursulfase for 12 months, and found that in some patients the ROM of some joints improved by 8–19°. In studies on children younger than 5 years of age with MPS II, joint mobility either stabilized or slightly improved during 1 year ERT [Alcalde-Martín et al., 2010]. Therefore, it appears that effects of

TABLE III. Active and Passive Joint ROM in the Lower Extremities at Baseline and After GET IT

| Patient no., norm value or parameter | Hip extension (°) | Hip flexion (°) | Knee extension (°) | Knee flexion (°) |
|---|---|---|---|---|
| Active joint ROM | | | | |
| Norm | 15 | 120 | 0 | 130 |
| Patient 1 | 0/0 | 120/120 | 10/20 | 130/130 |
| Patient 2 | 15/15 | 100/120* | 0/10 | 130/110 |
| Patient 3 | 15/15 | 50/100* | 0/0 | 60/70 |
| Patient 4 | 0/0 | 120/120 | 20/20 | 110/110 |
| Patient 5 | 10/15 | 100/100 | 0/0 | 130/130 |
| Patient 6 | 0/0 | 120/120 | 0/0 | 130/130 |
| Patient 7 | 0/0 | 120/120 | 0/0 | 130/130 |
| Mean | 5.7/6.4 | 104.3/114.3 | 4.3/7.1 | 117.1/115.7 |
| SD | 7.3/8.0 | 25.7/9.8 | 7.9/9.5 | 26.3/22.3 |
| *t*-Test *P*-value | 0.178 (n.s.) | 0.108 (n.s.) | 0.086 (n.s.) | 0.345 (n.s.) |
| Passive joint ROM | | | | |
| Norm | 20 | 130 | 0 | 140 |
| Patient 1 | 20/20 | 120/120 | 10/20 | 140/140 |
| Patient 2 | 15/15 | 120/120 | 0/0 | 140/130 |
| Patient 3 | 15/15 | 70/110* | 0/0 | 100/100 |
| Patient 4 | 0/0 | 120/120 | 20/20 | 60/70* |
| Patient 5 | 10/15 | 100/100 | 0/0 | 110/120* |
| Patient 6 | 20/20 | 130/130 | 0/0 | 140/140 |
| Patient 7 | 20/20 | 130/130 | 0/0 | 140/140 |
| Mean | 14.3/15.0 | 112.9/118.6 | 4.3/5.7 | 118.6/120.0 |
| SD | 7.3/7.1 | 21.4/10.7 | 7.9/9.8 | 30.8/26.5 |
| *t*-Test *P*-value | 0.178 (n.s.) | 0.178 (n.s.) | 0.178 (n.s.) | 0.302 (n.s.) |

n.s., Not significant.
Values before slash indicate results before GET IT, and values after slash those measured after GET IT. Asterisk indicates improvement after GET IT.

GET IT on joint motion are comparable to, or even somewhat more pronounced than those of ERT in MPS II patients (compare the results by others, mentioned above, with Tables II and III in this report).

In conclusion, we suggest that GET IT might potentially be tested as a therapy for MPS II either alone or in a combination with other treatments, like ERT. Moreover, results presented in this report support the proposal that SRT may be considered as an efficient procedure for treatment of various MPS disorders and other lysosomal storage diseases [Jakobkiewicz-Banecka et al., 2007; Dziedzic et al., 2010; Kaidonis et al., 2010]. Additional advantages of GET IT are: (i) oral administration, (ii) relatively low cost, (iii) no need of care at medical facility, and (iv) favorable safety profile (no significant adverse effects reported to date).

## ACKNOWLEDGMENTS

This work was supported by Ministry of Science and Higher Education of Poland (project grants no. N302 046 32/3603 to G.W. and 3631/B/P01/2007/33 to J.J.-B.), and was operated within the Foundation for Polish Science Team Programme co-financed by the EU European Regional Development Fund (grant no. TEAM/2008-2/7 to G.W.). The authors are grateful to Polish MPS Society for a general support.

## REFERENCES

Alcalde-Martín C, Muro-Tudelilla JM, Cancho-Candela R, Gutiérrez-Solana LG, Pintos-Morell G, Martí-Herrero M, Munguira-Aguado P, Galán-Gómez E. 2010. First experience of enzyme replacement therapy with idursulfase in Spanish patients with Hunter syndrome under 5 years of age: Case observations from the Hunter Outcome Survey (HOS). Eur J Med Genet 53:371–377.

Burrow TA, Leslie ND. 2008. Review of the use of idursulfase in the treatment of mucopolysaccharidosis II. Biologics 2:311–320.

Carrino DA, Sorrell JM, Caplan AI. 2000. Age-related changes in the proteoglycans of human skin. Arch Biochem Biophys 373:91–101.

Cox-Brinkman J, Smeulders MJ, Hollak CE, Wijburg FA. 2007. Restricted upper extremity range of motion in mucopolysaccharidosis type I: No response to one year of enzyme replacement therapy. J Inherit Metab Dis 30:47–50.

Dziedzic D, Węgrzyn G, Jakóbkiewicz-Banecka J. 2010. Impairment of glycosaminoglycan synthesis in mucopolysaccharidosis type IIIA cells by using siRNA: A potential therapeutic approach for Sanfilippo disease. Eur J Hum Genet 18:200–205.

Friso A, Tomanin R, Salvalaio M, Scarpa M. 2010. Genistein reduces glycosaminoglycan levels in a mouse model of mucopolysaccharidosis type II. Br J Pharmacol 159:1082–1091.

Gerhardt JJ, Rondinelli RD. 2001. Goniometric techniques for range-of-motion assessment. Phys Med Rehabil Clin N Am 12:507–527.

Jakobkiewicz-Banecka J, Wegrzyn A, Wegrzyn G. 2007. Substrate deprivation therapy: A new hope for patients suffering from neuronopathic forms of inherited lysosomal storage diseases. J Appl Genet 48:383–388.

Jakobkiewicz-Banecka J, Piotrowska E, Narajczyk M, Baranska S, Wegrzyn G. 2009. Genistein-mediated inhibition of glycosaminoglycan synthesis, which corrects storage in cells of patients suffering from mucopolysaccharidoses, acts by influencing an epidermal growth factor-dependent pathway. J Biomed Sci 16:26.

Kaidonis X, Liaw WC, Roberts AD, Ly M, Anson D, Byers S. 2010. Gene silencing of EXTL2 and EXTL3 as a substrate deprivation therapy for heparan sulphate storing mucopolysaccharidoses. Eur J Hum Genet 18:194–199.

Lim-Melia ER, Kronn DF. 2009. Current enzyme replacement therapy for the treatment of lysosomal storage diseases. Pediatr Ann 38:448–455.

Malinowska M, Jakobkiewicz-Banecka J, Kloska A, Tylki-Szymanska A, Czartoryska B, Piotrowska E, Wegrzyn A, Wegrzyn G. 2008. Abnormalities in the hair morphology of patients with some but not all types of mucopolysaccharidoses. Eur J Pediatr 167:203–209.

Malinowska M, Wilkinson FL, Bennett W, Langford-Smith KJ, O'Leary HA, Jakobkiewicz-Banecka J, Wynn R, Wraith JE, Wegrzyn G, Bigger BW. 2009. Genistein reduces lysosomal storage in peripheral tissues of mucopolysaccharide IIIB mice. Mol Genet Metab 98:235–242.

Murata K, Horiuchi Y. 1978. Age-dependent distribution of acidic glycosaminoglycans in human kidney tissue. Nephron 20:111–118.

Neufeld EF, Muenzer J. 2001. The mucopolysaccharidoses. In: Scriver CR, Beaudet AL, Sly WS, Valle D, editors. The metabolic and molecular bases of inherited disease, 8th edition. New York: McGraw-Hill Co. pp 3421–3452.

Okuyama T, Tanaka A, Suzuki Y, Ida H, Tanaka T, Cox GF, Eto Y, Orii T. 2010. Japan Elaprase Treatment (JET) study: Idursulfase enzyme replacement therapy in adult patients with attenuated Hunter syndrome (Mucopolysaccharidosis II, MPS II). Mol Genet Metab 99:18–25.

Olczyk K. 1993. Age-related changes in glycosaminoglycans of human intervertebral discs. Folia Histochem Cytobiol 31:215–220.

Pennock CA. 1976. A review and selection of simple laboratory methods used for the study of glycosaminoglycan excretion and the diagnosis of mucopolysaccharidoses. J Clin Pathol 29:111–123.

Piotrowska E, Jakobkiewicz-Banecka J, Baranska S, Tylki-Szymanska A, Czartoryska B, Wegrzyn A, Wegrzyn G. 2006. Genistein-mediated inhibition of glycosaminoglycan synthesis as a basis for gene expression-targeted isoflavone therapy for mucopolysaccharidoses. Eur J Hum Genet 14:846–852.

Piotrowska E, Jakobkiewicz-Banecka J, Tylki-Szymanska A, Liberek A, Maryniak A, Malinowska M, Czartoryska B, Puk E, Kloska A, Liberek T, Baranska S, Wegrzyn A, Wegrzyn G. 2008. Genistin-rich soy isoflavone extract in substrate reduction therapy for Sanfilippo syndrome: An open-label, pilot study in 10 pediatric patients. Curr Ther Res Clin Exp 63:166–179.

Piotrowska E, Jakóbkiewicz-Banecka J, Maryniak A, Tylki-Szymańska A, Puk E, Liberek A, Węgrzyn A, Czartoryska B, Somińska-Wojewódzka M, Węgrzyn G. 2011. Two years follow-up of Sanfilippo disease patients treated with a genistein-rich isoflavone extract: Assessment of effects on cognitive functions and general status of patients. Med Sci Monit 17:CR196–202.

Rohrbach M, Clarke JT. 2007. Treatment of lysosomal storage disorders: Progress with enzyme replacement therapy. Drugs 67:2697–2716.

Tomatsu S, Gutierrez MA, Ishimaru T, Peña OM, Montaño AM, Maeda H, Velez-Castrillon S, Nishioka T, Fachel AA, Cooper A, Thornley M, Wraith E, Barrera LA, Laybauer LS, Giugliani R, Schwartz IV, Frenking GS, Beck M, Kircher SG, Paschke E, Yamaguchi S, Ullrich K, Isogai K, Suzuki Y, Orii T, Noguchi A. 2005. Heparan sulfate levels in mucopolysaccharidoses and mucolipidoses. J Inherit Metab Dis 28:743–757.

Tylki-Szymanska A, Marucha J, Jurecka A, Syczewska M, Czartoryska B. 2010a. Efficacy of recombinant human alpha-L-iduronidase (laronidase) on restricted range of motion of upper extremities in mucopolysaccharidosis type I patients. J Inherit Metab Dis 33:151–157.

Tylki-Szymanska A, Rozdzynska A, Jurecka A, Marucha J, Czartoryska B. 2010b. Anthropometric data of 14 patients with mucopolysaccharidosis I: Retrospective analysis and efficacy of recombinant human alpha-L-iduronidase (laronidase). Mol Genet Metab 99:10–17.

Voznyi YV, Keulemans JL, van Diggelen OP. 2001. A fluorimetric enzyme assay for the diagnosis of MPS II (Hunter disease). J Inherit Metab Dis 24:675–680.

Wegrzyn G, Jakobkiewicz-Banecka J, Gabig-Ciminska M, Piotrowska E, Narajczyk M, Kloska A, Malinowska M, Dziedzic D, Golebiewska I, Moskot M, Wegrzyn A. 2010. Genistein: A natural isoflavone with a potential for treatment of genetic diseases. Biochem Soc Trans 38:695–701.

Wraith JE, Scarpa M, Beck M, Bodamer OA, De Meirleir L, Guffon N, Meldgaard Lund A, Malm G, Van der Ploeg AT, Zeman J. 2008. Mucopolysaccharidosis type II (Hunter syndrome): A clinical review and recommendations for treatment in the era of enzyme replacement therapy. Eur J Pediatr 167:267–277.