Adverse Effects of Genistein in a Mucopolysaccharidosis Type I Mouse Model

Sandra D.K. Kingma · Tom Wagemans ·
Lodewijk IJlst · Jurgen Seppen · Marion J.J. Gijbels ·
Frits A. Wijburg · Naomi van Vlies

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Abstract Mucopolysaccharidosis type I (MPS I) is a lysosomal storage disorder characterized by diminished degradation of the glycosaminoglycans heparan sulfate (HS) and dermatan sulfate (DS). Patients present with a variety of symptoms, including severe skeletal disease. Current therapeutic strategies have only limited effects on bone disease. The isoflavone genistein has been studied as a potential therapy for the mucopolysaccharidoses because of its putative ability to inhibit GAG synthesis and subsequent accumulation. Cell, animal, and clinical studies, however, showed variable outcomes. To determine the effects of genistein on MPS I-related bone disease, wild-type (WT) and MPS I mice were fed a genistein-supplemented diet (corresponding to a dose of approximately 160 mg/kg/day) for 8 weeks. HS and DS levels in bone and plasma

remained unchanged after genistein supplementation, while liver HS levels were decreased in genistein-fed MPS I mice as compared to untreated MPS I mice. Unexpectedly, genistein-fed mice exhibited significantly decreased body length and femur length. In addition, 60% of genistein-fed MPS I mice developed a scrotal hernia and/or scrotal hydrocele, manifestations which were absent in WT or untreated MPS I mice. In contrast to studies in MPS III mice, our study in MPS I mice demonstrates no beneficial but even potential adverse effects of genistein supplementation. Our results urge for a cautious approach on the use of genistein, at least in patients with MPS I.

Introduction

Mucopolysaccharidosis type I (MPS I, OMIM 252800) is a lysosomal storage disease (LSD) caused by α -L-iduronidase (IDUA, EC 3.2.1.76) deficiency, resulting in impaired degradation and subsequent accumulation of the glycosaminoglycans (GAGs) heparan sulfate (HS) and dermatan sulfate (DS). Patients may present with cardiac and pulmonary disease, inguinal and umbilical hernias, corneal clouding, and progressive central nervous system (CNS) disease which significantly limits life expectancy (Muenzer 2011). In addition, skeletal dysplasia, generally referred to as dysostosis multiplex, is a striking feature and a major cause of morbidity. Patients show progressive loss of range of joint motion with contractures, growth arrest, kyphosis, scoliosis, hip dysplasia, and hypoplastic vertebral bodies resulting in spinal cord compression (Muenzer 2011; White 2011). Current therapies, including hematopoietic stem cell transplantation (HSCT) and enzyme replacement therapy, effectively treat many features of MPS I but have limited effects on bone disease (Weisstein et al. 2004; Sifuentes et al. 2007).

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S.D.K. Kingma · T. Wagemans · F.A. Wijburg (✉) · N. van Vlies
Department of Pediatrics and Amsterdam Lysosome Centre “Sphinx”,
Academic Medical Center, University of Amsterdam, Amsterdam,
The Netherlands
e-mail: f.a.wijburg@amc.uva.nl

S.D.K. Kingma · T. Wagemans · L. IJlst · N. van Vlies
Laboratory of Genetic Metabolic Diseases, Department of Clinical
Chemistry and Pediatrics, Academic Medical Center, University of
Amsterdam, Amsterdam, The Netherlands

J. Seppen
Tytgat Institute for Liver and Intestinal Research, Academic Medical
Center, University of Amsterdam, Amsterdam, The Netherlands

M.J.J. Gijbels
Department of Medical Biochemistry, Academic Medical Center,
University of Amsterdam, Amsterdam, The Netherlands

M.J.J. Gijbels
Department of Molecular Genetics and Department of Pathology,
Cardiovascular Research Institute Maastricht, Maastricht, The
Netherlands

Several factors contribute to this lack of effect. Firstly, the inability of the relatively large enzyme to traffic through the poorly vascularized matrix of growth plates and other cartilaginous tissue to target cells (Aldenhoven et al. 2009; Baldo et al. 2013). Secondly, cartilage cells are derived from mesenchymal stem cells, which are not transplanted in sufficient amounts by HSCT (Koç et al. 2002). Thirdly, therapy is started after the onset of irreversible bone lesions, which may already exist before birth (Martin and Ceuterick 1983; Hinek and Wilson 2000). An alternative treatment strategy for LSDs is substrate reduction therapy, which aims to reduce the synthesis of the accumulating material, thereby preventing or halting lysosomal storage. This approach has been used successfully in Gaucher disease and Niemann–Pick disease type C (Hollak and Wijburg 2014).

The isoflavone genistein has several biological activities. It is an antioxidant, has estrogenic activity, and inhibits the activity of tyrosine kinase receptors including the epidermal growth factor receptor (EGFR) (Akiyama et al. 1987; Dixon and Ferreira 2002). Genistein has been shown to reduce GAG synthesis in MPS fibroblasts, at least partly via the latter mechanism (Jakóbkiewicz-Banecka et al. 2009). An in vivo study with a high dose of genistein in MPS IIIB mice showed reduced GAG levels in brain and impressive amelioration of neurological symptoms (Malinowska et al. 2010). Although genistein appears to be well tolerated in high doses, adverse effects, which are associated with its potential antiproliferative and estrogenic actions, have been reported and may include hepatotoxicity and hormonal disbalance (Kim et al. 2013; Singh et al. 2014). The only study on the effects of genistein on MPS-related bone disease showed increased range of joint motion in genistein-treated MPS II patients, suggesting that genistein at least reaches the surrounding connective and muscle tissue of the joints (Marucha et al. 2011). Because genistein can reach the bone tissue (Coldham and Sauer 2000), we fed MPS I mice a high-dose genistein diet to evaluate its potential for the treatment of MPS I-related bone disease.

Materials and Methods

Animals

MPS I mice (B6.129-*Idua*^{tm1Clk}/J, (Clarke et al. 1997)) were purchased from Jackson Laboratory and maintained as a heterozygote line on an inbred C57BL/6J background at the Academic Medical Center. The mice were housed at 21 ± 1°C, 40–50% humidity, on a 12 h light–dark cycle, with ad libitum access to water and food. Genotypes were identified by PCR using a protocol provided by Jackson Laboratory.

An AIN93M diet (Reeves 1997) with 3.7% sunflower oil and 0.3% rapeseed oil instead of 4% soy oil was produced (Research Diet Services BV). For the genistein-fed group, genistein aglycone (kind gift from Axcentua) was added to the diet in a concentration of 0.1% (w/w). Assuming a food intake of 0.16 g food per gram of body weight per day, this diet results in a dose of 160 mg/kg/day, which is similar to other studies on the effects of a high dose of genistein in MPS mice (Malinowska et al. 2009, 2010). One week before weaning, mice received the soy-free diet. At 3 weeks of age, male wild-type (WT) and MPS I mice were weaned on the soy-free diet or the genistein-supplemented diet ($n = 10$ per group). Every week, mice were weighed and examined for general pathological manifestations. If scrotal abnormalities were observed, scrotal hydrocele and/or scrotal hernias were objectified by macroscopic inspection, transillumination, and/or ultrasound by a skilled animal technician and macroscopic inspection of the scrotum and abdominal and pelvic cavity after sacrifice. At 11 weeks of age, mice were anesthetized with an intraperitoneal injection of 100 mg/kg pentobarbital and euthanized by exsanguination via cardiac puncture. Body length and femur length were measured, and blood and tissues collected.

All animal experiments were approved by the animal institutional review board at the Academic Medical Center, University of Amsterdam.

Tissue Processing

Blood was collected into EDTA tubes, kept on ice, and centrifuged at 240 g for 10 min. Plasma was collected and stored at −80°C until analysis. Mouse livers were snap-frozen in liquid nitrogen and stored at −80°C. Before analysis, mouse livers were homogenized in PBS, and protein concentration was determined using Pierce[®] BCA Protein Assay Reagent A (Thermo Scientific) as described by the manufacturer. Mouse humeri were collected and placed in 0.9% NaCl containing complete mini protease inhibitor cocktail (Roche Applied Science) at 4°C for >1 day. Next, soft tissue was removed, and humeri were stored at −80°C. Before analysis, humeri were homogenized in PBS, sonicated twice for 15 s on 40 joules/watt/second using a Vibra Cell sonicator (Sonics & Materials Inc.), and centrifuged for 1 min at 400 g. Protein concentration of the supernatant was determined as described above.

GAG Analysis

GAG levels in mouse plasma, liver, and humerus homogenates were determined using HPLC-MS/MS, as described previously (de Ru et al. 2013), with one modification for tissue samples: 12.5 µg protein of liver or humerus

homogenates were used. Genistein supplementation did not alter HS or DS disaccharide composition; therefore, only values of the most abundant HS derived disaccharide D0A0 and DS derived disaccharide D0a4 are given.

Genistein Measurement

Genistein levels were determined by ultrahigh performance liquid chromatography (UHPLC) with a protocol modified from Seppen et al. (2012). 40 μ l of plasma or humerus homogenate was acidified with 5 μ l 1M 4-morpholineethanesulfonic acid. Genistein was hydrolyzed by adding 2.5 μ l β -glucuronidase (Sigma Aldrich) and 2.5 μ l sulfatase (10 mg/ml in PBS, Sigma Aldrich) and incubated at 37°C for 20 h. Protein was precipitated by adding 100 μ l methanol and samples were centrifuged at 8,000g for 2 min. The supernatant was vaporized using a Techne® Dri-block® heater (Bibby Scientific) and the residue dissolved in 20% v/v acetonitrile.

UHPLC was performed on a Dionex UltiMate 3000 UHPLC, equipped with a PolarAdvantage C18 column (Thermo Scientific), variable wavelength detector set at 260 nm, 5 μ l injection, and a flow rate of 0.5ml/min. The following program was used with solvent A (A, 0.1% v/v formic acid in water) and solvent B (B, 0.1% v/v formic acid in acetonitrile): start 30% B, $t = 5$ 55% B, $t = 5.1$ 100% B, $t = 6.1$ 30% B, and $t = 10$ 30% B. Linear gradients were employed and t is in minutes. Dionex Chromeleon software (Thermo Scientific) was used to integrate the chromatograms, and genistein level was calculated using a calibration curve of genistein dissolved in dimethyl sulfoxide, diluted in mouse plasma.

Morphology of Testes

The morphology of testicular cells and structures was analyzed by routine hematoxylin/eosin (HE) staining.

Statistical Analysis

Statistical analysis was performed using Mann–Whitney U tests for nonparametric analysis with SPSS Statistics software version 21 (IBM Corp.). Significance was assumed where p values were <0.05 .

Results

Genistein Decreases GAG Levels in the Liver but Not in the Bone or Plasma

As expected, analysis of GAG levels revealed significantly higher levels in MPS I plasma and tissues ($p < 0.0001$),

Fig. 1 GAG levels. GAG levels in the bone (a), plasma (b), and liver (c) in control and genistein-supplemented mice. The results are expressed as milligrams GAGs per gram of protein for the liver and bone or as milligrams GAGs per liter of plasma. All values are mean $\pm$ standard deviation of 10 mice. Each sample was analyzed in duplicate, ** $p < 0.01$, ND not detectable, G genistein supplementation

as compared to WT (Fig. 1). Genistein supplementation did not affect GAG levels in WT or MPS I bone or plasma (Fig. 1a, b). HS levels in the liver from genistein-fed MPS I mice were 25% decreased ($p < 0.01$), as compared to untreated mice. No difference in liver DS levels was observed between genistein-fed and untreated MPS I mice (Fig. 1c).

Genistein Was Present in the Plasma but Undetectable in the Bone

Genistein levels in the plasma of genistein-fed MPS I mice were 782 nM $\pm$ 503. In the control group, genistein concentration in the plasma was below the limit of quantification (data not shown). Genistein levels in the bone were below detection level in all MPS I mice (data not shown).

Genistein Causes Decreased Skeletal Growth and Scrotal Hernia/Hydrocele in MPS I Mice

Total body weight of MPS I mice was 11% increased ($p < 0.05$), as compared to WT mice (Fig. 2a). Total body weight of genistein-fed MPS I mice was decreased by 16% ($p < 0.001$) (Fig. 2a), as compared to untreated MPS I mice. Following genistein supplementation, total body length was 4% ($p < 0.05$) and 6% ($p < 0.001$) decreased in WT and MPS I mice (Fig. 2b), respectively, as compared to untreated mice. Femur length of MPS I mice was 4% decreased ($p < 0.05$), as compared to WT mice (Fig. 2c). Femur length of genistein-fed mice were 3% ($p < 0.01$) and 7% ($p < 0.001$) decreased in WT and MPS I mice (Fig. 2c), respectively, compared to untreated mice. Surprisingly, 60% of the genistein-fed MPS I mice had an enlarged scrotum (Fig. 3) with redness of the overlying skin, and the mice showed a wide-based gait. None of the WT or untreated MPS I mice showed scrotal abnormalities. The scrotal enlargements were observed to be due to either scrotal hernia and/or scrotal hydrocele. HE staining of testes revealed no morphological changes of testicular cells and structures in genistein-fed or untreated mice (results not shown).

Discussion

Skeletal disease is one of the most prevalent and incapacitating disease manifestations in patients suffering from the MPSs and frequently results in the need for multiple surgical interventions (Muenzer 2011; White 2011). Current disease-modifying therapies for the management of MPS I ameliorate a number of clinical signs and symptoms but have a limited effect on the progression of skeletal disease (Weisstein et al. 2004; Sifuentes et al. 2007). Therefore, therapeutic strategies targeting bone disease are urgently needed.

The isoflavone genistein inhibits the activity of tyrosine kinase receptors, thereby modulating the expression of several genes, including some involved in GAG synthesis (Akiyama et al. 1987; Moskot et al. 2014). Genistein is being investigated for its potential benefit in the treatment

Fig. 2 Weight, body length, and femur length. Weight (a), body length (b), and femur length (c) in control and genistein-supplemented mice. All values are mean $\pm$ standard deviation of 10 mice, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, G genistein supplementation

of CNS disease in MPS III, as it passes the blood–brain barrier, and an in vivo study showed that genistein may reduce GAG accumulation in the brain and corrects behavioral abnormalities in MPS III mice (Tsai 2005;

Fig. 3 Scrotal abnormalities. Scrotum of a MPS I mouse on control diet (**a**) and a MPS I mouse on genistein diet (**b**). 60% of MPS I mice with genistein had either scrotal hydrocele or scrotal hernia, as depicted in (**b**). All other mice had scrota as depicted in (**a**)

Malinowska et al. 2010; Langford-Smith et al. 2011). As prevention of GAG accumulation in the cartilage and bone might halt the progression of skeletal disease in the MPSs, we investigated the effects of genistein on GAG accumulation in MPS I mice in order to evaluate its potential for the treatment of MPS I-related bone disease.

In agreement with results of previously published studies on the effects of genistein in MPS mice (Malinowska et al. 2009; Friso et al. 2010), GAG levels were significantly decreased in the liver of genistein-fed MPS I mice. Surprisingly, we observed no effect on GAGs in the bone or plasma. The effect of genistein on GAG levels in the bone or plasma of MPS III mice has not been described in previous studies (Malinowska et al. 2009, 2010; Friso et al. 2010). In rats, genistein is known to reach bone in approximately 17 times lower quantities when compared to plasma levels, 2 h after administration of 4 mg/kg genistein (Coldham and Sauer 2000) (in the current study, we used 160 mg/kg/day oral supplementation). Although plasma genistein concentrations in our study were compa-

rable with or even higher than the concentrations reported in other studies on the effects of genistein in mice (Santell et al. 2000; Mentor-Marcel et al. 2001), genistein concentrations in the bone in our study were below detection level. These results suggest that the treatment duration and/or dosage of genistein may have been insufficient to treat the bone. In our study, mice were treated from 3 weeks of age, for a period of 8 weeks as, at 11 weeks of age, the growth plate is almost closed and skeletal development almost completed. Therefore, we do not expect any additional effect on the bone with a longer supplementation period. Earlier initiation of genistein supplementation is not feasible as mice are weaned at 3 weeks of age.

Our observation that genistein supplementation led to decreased body weight in mice was not surprising. Although not decreased previously in MPS III mice (Malinowska et al. 2009, 2010; Friso et al. 2010), the effect of genistein on decreasing lipid deposition and body weight has been reported earlier in mice and rats and may be due to the estrogenic effect of increasing lipoprotein lipase activity (Naaz et al. 2003) or decreased food intake (Seppen 2012). Genistein-fed mice in our study, however, also exhibited decreased skeletal growth, including decreased femur length, which has not been described previously. In addition, 60% of genistein-fed MPS I mice developed a scrotal hernia and/or a scrotal hydrocele. The cause of the decrease in skeletal growth and the observed scrotal hernia/hydroceles is unclear. Increased levels of DS, an abundant GAG in connective tissue, may have contributed to the development of adverse effects, as our recently published study showed that genistein can increase HS and DS storage in MPS I chondrocytes and fibroblasts (but not in osteoblasts) (Kingma et al. 2014). In the present study, GAG levels were analyzed in complete bones, and chondrocytes only make up a small portion of the entire bone. This might explain the fact that no increase in GAG levels was observed in the bones of genistein-treated MPS I mice, while these animals did show a decrease in femur length. Increased GAG storage in certain cell types might further stimulate the pathophysiological mechanisms causing bone and connective tissue disease in MPS I. In addition, patients with MPS I already frequently display hernias and hydroceles (Arn et al. 2009). Genistein may thus lead to a more severe MPS I phenotype, at least in mice. Therefore, increasing the dose of genistein or prolonging the supplementation period likely will not result in amelioration of bone disease in MPS I mice. The only study in patients on the effects of a high dose, i.e., 150 mg/kg/day, of (synthetic) genistein (at least 1-year treatment of 22 MPS III patients) concluded that genistein is safe but did report elevation of liver enzymes, breast development in

boys or young girls, menstrual irregularities, and bilateral deep vein thrombosis in a 20-year-old woman with several other risk factors for thrombosis (Kim et al. 2013). This study did not report on growth rate.

In conclusion, our study suggests that high doses of genistein might lead to decreased growth and increased incidence of scrotal hydroceles and hernias, at least in MPS I. These data, in combination with other potential adverse effects reported in previous studies (Kim et al. 2013; Singh et al. 2014), support a cautious approach to the introduction of high doses of genistein in patients with various MPSs and underscores the need for well-designed and controlled clinical trials allowing the collection of all potential adverse effects over longer periods of time.

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Take-Home Message

Genistein-fed mucopolysaccharidosis type I mice exhibit scrotal hernias/hydroceles and decreased skeletal growth, emphasizing the need for caution when using genistein in patients with MPS.

Compliance with Ethics Guidelines

Conflicts of Interest

Sandra Kingma, Tom Wagemans, Lodewijk IJlst, Jurgen Seppen, Marion Gijbels, Frits Wijburg, and Naomi van Vlies declare that they have no conflicts of interest.

Informed Consent

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

Animal Rights

All institutional and national guidelines for the care and use of laboratory animals were followed.

Contributions of the Individual Authors

Sandra D.K. Kingma: designing, conducting, reporting, and revising the work described in the article.

Tom Wagemans: conducting the work described in the article.

Lodewijk IJlst: designing and revising the work described in the article.

Jurgen Seppen: conducting and revising the work described in the article.

Marion J.J. Gijbels: conducting and revising the work described in the article.

Frits A. Wijburg: designing, reporting, and revising the work described in the article.

Naomi van Vlies: designing, reporting, and revising the work described in the article.

References

- Akiyama T, Ishida J, Nakagawa S et al (1987) Genistein, a specific inhibitor of tyrosine-specific protein kinases. *J Biol Chem* 262: 5592–5595
- Aldenhoven M, Sakkers RJB, Boelens J, de Koning TJ, Wulffraat NM (2009) Musculoskeletal manifestations of lysosomal storage disorders. *Ann Rheum Dis* 68:1659–1665
- Am P, Wraith JE, Underhill L (2009) Characterization of surgical procedures in patients with mucopolysaccharidosis type I: findings from the MPS I Registry. *J Pediatr* 154:859–64.e3
- Baldo G, Mayer FQ, Martinelli BZ et al (2013) Enzyme replacement therapy started at birth improves outcome in difficult-to-treat organs in mucopolysaccharidosis I mice. *Mol Genet Metab* 109: 33–40
- Clarke LA, Russell CS, Pownall S et al (1997) Murine mucopolysaccharidosis type I: targeted disruption of the murine alpha-L-iduronidase gene. *Hum Mol Genet* 6:503–511
- Coldham NG, Sauer MJ (2000) Pharmacokinetics of [(14)C]Genistein in the rat: gender-related differences, potential mechanisms of biological action, and implications for human health. *Toxicol Appl Pharmacol* 164:206–215
- De Ru MH, van der Tol L, van Vlies N et al (2013) Plasma and urinary levels of dermatan sulfate and heparan sulfate derived disaccharides after long-term enzyme replacement therapy (ERT) in MPS I: correlation with the timing of ERT and with total urinary excretion of glycosaminoglycans. *J Inher Metab Dis* 36: 247–255
- Dixon RA, Ferreira D (2002) Genistein. *Phytochemistry* 60:205–211
- 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
- Hinek A, Wilson SE (2000) Impaired elastogenesis in Hurler disease: dermatan sulfate accumulation linked to deficiency in elastin-binding protein and elastic fiber assembly. *Am J Pathol* 156: 925–938
- Hollak CEM, Wijburg FA (2014) Treatment of lysosomal storage disorders: successes and challenges. *J Inher Metab Dis* 37: 587–598
- Jakóbkiewicz-Banecka J, Piotrowska E, Narajczyk M, Barańska S, Węgrzyn 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
- Kim KH, Dodsworth C, Paras A, Burton BK (2013) High dose genistein aglycone therapy is safe in patients with mucopolysaccharidoses involving the central nervous system. *Mol Genet Metab* 109:382–385

- Kingma SDK, Wagemans T, IJlst L, Wijburg FA, van Vlies N (2014) Genistein increases glycosaminoglycan levels in mucopolysaccharidosis type I cell models. *J Inherit Metab Dis* 37:813–821
- Koç ON, Day J, Nieder M, Gerson SL, Lazarus HM, Krivit W (2002) Allogeneic mesenchymal stem cell infusion for treatment of metachromatic leukodystrophy (MLD) and Hurler syndrome (MPS-IH). *Bone Marrow Transplant* 30:215–222
- Langford-Smith A, Malinowska M, Langford-Smith KJ et al (2011) Hyperactive behaviour in the mouse model of mucopolysaccharidosis IIIB in the open field and home cage environments. *Genes Brain Behav* 10:673–682
- Malinowska M, Wilkinson FL, Bennett W et al (2009) Genistein reduces lysosomal storage in peripheral tissues of mucopolysaccharide IIIB mice. *Mol Genet Metab* 98:235–242
- Malinowska M, Wilkinson FL, Langford-Smith KJ et al (2010) Genistein improves neuropathology and corrects behaviour in a mouse model of neurodegenerative metabolic disease. *PLoS One* 5:e14192
- Martin JJ, Ceuterick C (1983) Prenatal pathology in mucopolysaccharidoses: a comparison with postnatal cases. *Clin Neuropathol* 2:122–127
- 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 A* 155A:2257–2262
- Mentor-Marcel R, Lamartiniere CA, Eltoum I, Greenberg NM, Elgavish A (2001) Genistein in the Diet Reduces the Incidence of Poorly Differentiated Prostatic Adenocarcinoma in Transgenic Mice (TRAMP). *Cancer Res* 61:6777–6782
- Moskot M, Montefusco S, Jakóbkiewicz-Banecka J et al (2014) The phytoestrogen genistein modulates lysosomal metabolism and transcription factor EB (TFEB) activation. *J Biol Chem* 289:17054–17069
- Muenzer J (2011) Overview of the mucopolysaccharidoses. *Rheumatology (Oxford, England)* 50(Suppl 5):v4–v12
- Naaz A, Yellayi S, Zakroczyński M (2003) The soy isoflavone genistein decreases adipose deposition in mice. *Endocrinology* 144:3315–3320
- Reeves PG (1997) Components of the AIN-93 diets as improvements in the AIN-76A diet. *J Nutr* 127:838S–841S
- Santell RC, Kieu N, Helferich WG (2000) Genistein inhibits growth of estrogen-independent human breast cancer cells in culture but not in athymic mice. *J Nutr* 130:1665–1669
- Seppen J (2012) A diet containing the soy phytoestrogen genistein causes infertility in female rats partially deficient in UDP glucuronyltransferase. *Toxicol Appl Pharmacol* 264:335–342
- Sifuentes M, Doroshow R, Hoft R et al (2007) A follow-up study of MPS I patients treated with laronidase enzyme replacement therapy for 6 years. *Mol Genet Metab* 90:171–180
- Singh P, Sharma S, Rath SK (2014) Genistein induces deleterious effects during its acute exposure in Swiss mice. *Biomed Res Int* 2014:619617
- Tsai T-H (2005) Concurrent measurement of unbound genistein in the blood, brain and bile of anesthetized rats using microdialysis and its pharmacokinetic application. *J Chromatogr A* 1073:317–322
- Weinstein JS, Delgado E, Steinbach LS, Hart K, Packman S (2004) Musculoskeletal manifestations of Hurler syndrome: long-term follow-up after bone marrow transplantation. *J Pediatr Orthop* 24:97–101
- White KK (2011) Orthopaedic aspects of mucopolysaccharidoses. *Rheumatology* 50(Suppl 5):v26–v33