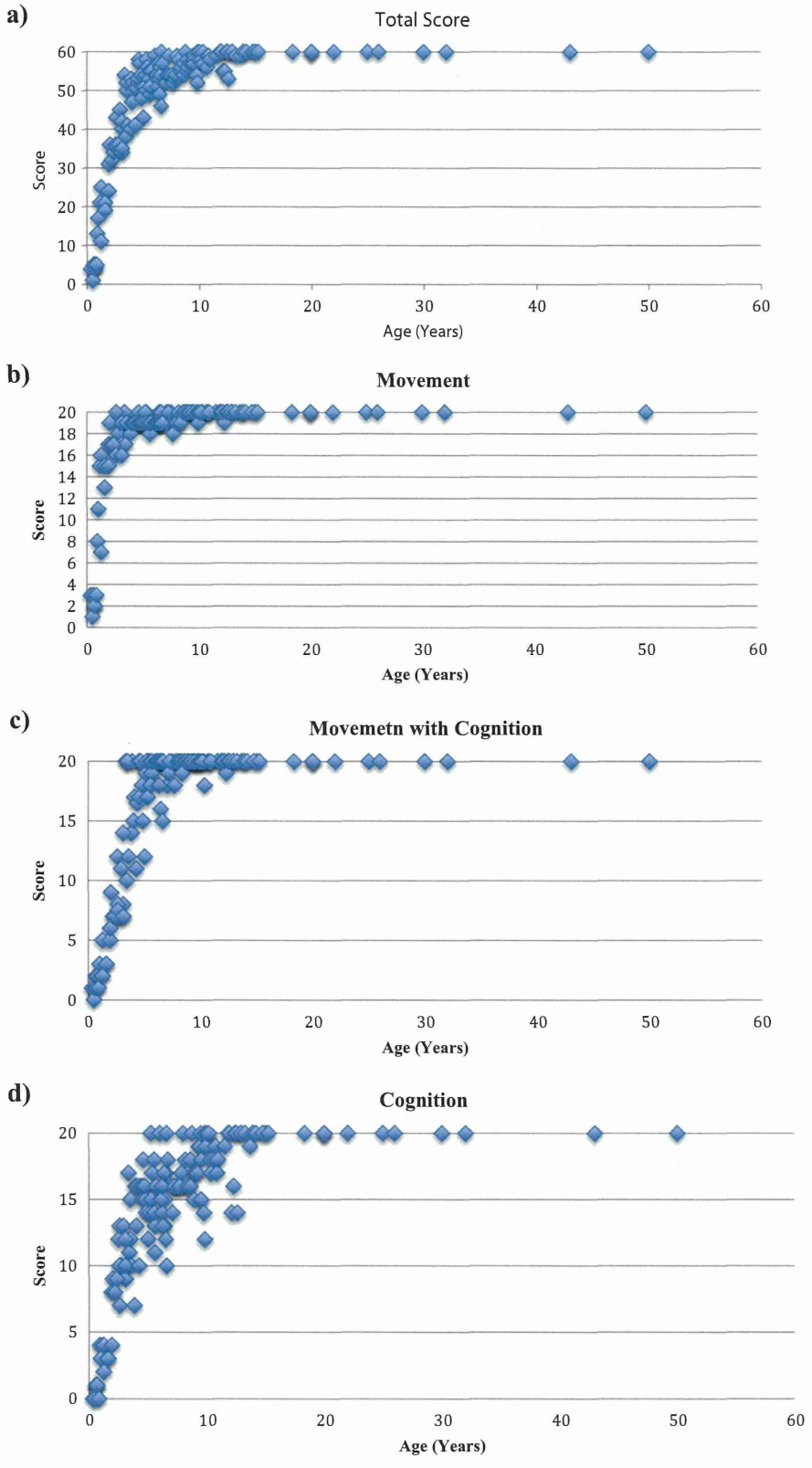

Fig. 2. ADL score for control subjects ($n = 138$; 4 months–50 years). (a) Total score, (b) "movement," (c) "movement with cognition," and (d) "cognition."

3.4. ADL scores in ERT vs HSCT patients

Table 3 shows a comparison of severe phenotypic patients who had been given HSCT, early ERT, or late ERT. In patients with a severe phenotype treated by HSCT ($n = 18$; mean present age 12.69 ± 5.17 years), the overall average score was 27.9 ± 11.4, and scores were similar in all age-groups. Lower scores were generally seen in toileting, conversation, and problem solving subcategories (Fig. 4).

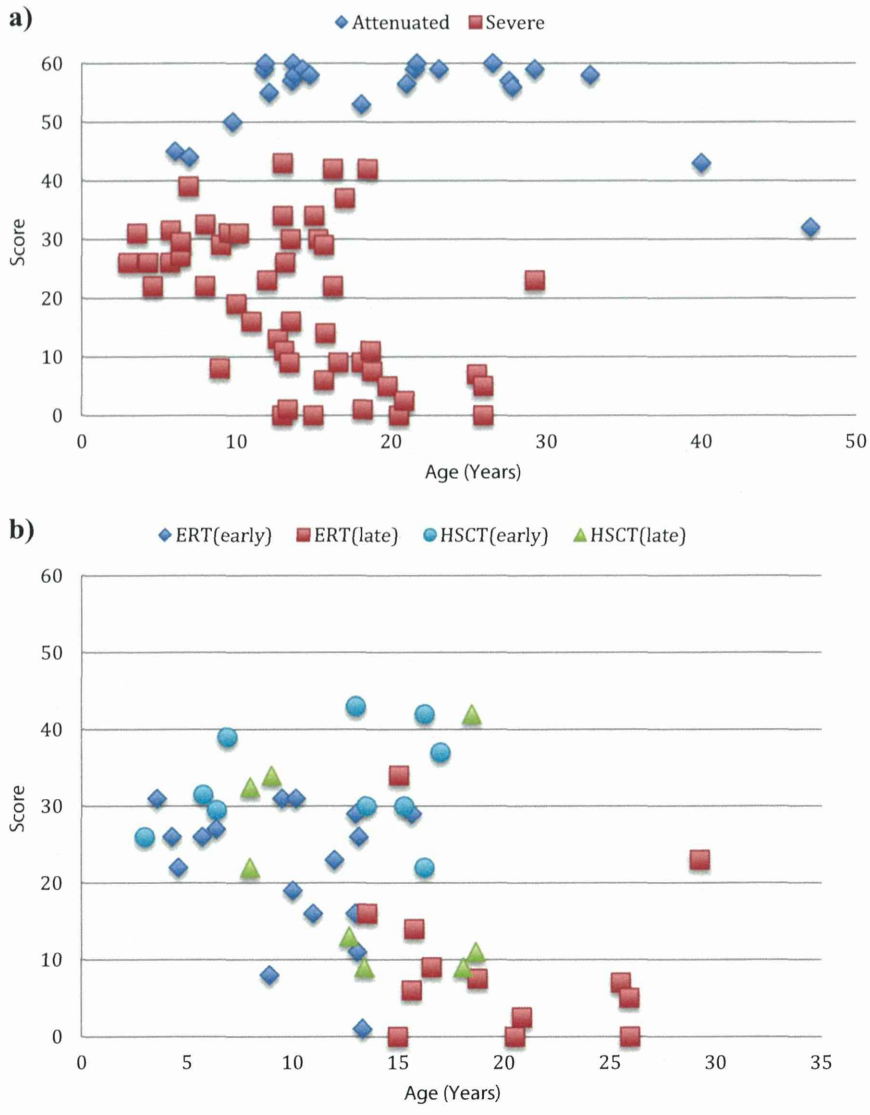

Fig. 3. ADL score for patients with Hunter syndrome. (a) Total ADL score for patients with severe ($n = 50$; 3 years–29 years) or attenuated ($n = 23$; 6 years–47 years) phenotypes. (b) Total ADL score of patients with severe phenotypes by treatment type. Early ERT ($n = 16$); late ERT ($n = 13$); early HSCT ($n = 10$); late HSCT ($n = 8$).

For severe phenotypic patients who started ERT at an early age ($n = 16$; mean present age 10.1 ± 3.7 years), the overall average score was 21.6 ± 9.0. Average scores in each domain and age-group were lower than in the HSCT group, but were not statistically different.

Late ERT patients ($n = 13$; mean present age 19.8 ± 5.2 years) had lower scores than the HSCT and early ERT treatment groups (mean score 9.5 ± 10.5, Table 3). Nine out of 13 (70%) patients had less than 10 points.

The five highest scores among treated groups were obtained by HSCT-treated patients while the lowest score (0) was obtained by three late ERT patients (Fig. 3b). The highest scores for both treatment types were seen in hand movement and walking (Fig. 4).

3.5. Early treatment with HSCT

The early HSCT group, treated before 5 years of age ($n = 10$; mean age at analysis 11.3 ± 5.2 years), scored 33 ± 7.0 while late HSCT group ($n = 8$; mean age 13.3 ± 4.7 years) scored 21.2 ± 13.0 ($p < 0.05$). The 4 HSCT patients with less than 20 points were in the late HSCT group. Scores in all three domains were higher in the early HSCT group than those in the late HSCT group: "movement" (17.3 ± 2.8 vs 12.4 ± 6.2), "movement with cognition" (10.9 ± 4.1 vs 5.8 ± 3.4), and "cognition" (4.8 ± 2.0 vs 3.4 ± 3.9). Two patients diagnosed as severe phenotype Hunter syndrome were treated with HSCT at 2 years of age. They were 3 and 17 years old at the time of this study. The 3-year-old patient obtained a score of 26, relatively normal compared to age-matched controls. The 17-year-old patient obtained a score of 42, which is among the highest for patients with a severe phenotype.

4. Discussion

Hunter syndrome is a progressive systemic disease of the CNS and the skeleton that has a serious impact on the daily activities of patients and their families. Therefore, characterizing the ADL will provide a better understanding of the obstacles for patients with Hunter syndrome, thereby enabling physicians to evaluate therapeutic efficacy and parents/families to understand the progression of the patient's quality of life.

Traditional questionnaires such as the FIM, HS-FOCUS, and PEDI have been used to evaluate ADL for patients with Hunter syndrome

Table 2
ADL scores for age-matched controls, patients with attenuated phenotypes, and patients with severe phenotypes.

		Subject age range (years)			
		0–5	5–10	10–15	>15
Control	*n*	46	54	25	13
	Movement	14.96 ± 6.10	19.43 ± 0.57	19.96 ± 0.20	20
	Movement cognition	9.46 ± 6.71	19.38 ± 1.47	19.88 ± 0.44	20
	Cognition	8.87 ± 4.63	16.07 ± 1.80	18.76 ± 1.37	20
Severe	*n*	4	10	15	21
	Movement	15.75 ± 2.22	15.25 ± 4.44	10.07 ± 5.11	7.31 ± 7.12
	p^a	NS	0.0155	9.01E−05	8.53E−08
	Movement cognition	6.5 ± 2.1	7.90 ± 2.83	5.79 ± 4.33	5.86 ± 6.05
	p^a	NS	8.27E−08	1.62E−08	9.88E−10
	Cognition	4 ± 2.45	4.4 ± 2.11	2.93 ± 2.95	3.21 ± 3.20
	p^a	0.0128	3.58E−10	1.97E−13	3.07E−16
Attenuated	*n*	–	4	8	13
	Movement	–	17.67 ± 1.52	19.5 ± 0.76	18.73 ± 3.26
	p^a	–	NS	NS	0.0498
	Movement cognition	–	18.66 ± 1.15	19.5 ± 1.07	17.63 ± 5.62
	p^a	–	NS	NS	NS
	Cognition	–	10 ± 1.73	19.25 ± 1.16	16.92 ± 5.89
	p^a	–	1.80E−02	NS	1.32E−03
t-test between phenotypes	Movement	–	NS	1.74E−05	2.20E−06
	Movement with cognition	–	5.24E−06	6.89E−09	6.33E−06
	Cognition	–	9.54E−03	2.47E−13	3.88E−08

[a]Student's *t*-test between phenotype and control; NS: no statistical significance at $p < 0.05$.

[16–18]. These questionnaires are limited by their age requirements, time consumption, complexity, and the requirement for professional examiners. We have developed a new questionnaire to characterize ADL in patients with Hunter syndrome. This new questionnaire, containing just 12 items total, is concise and simple enough so that patients and their families can self-assess without the need of a trained individual. The questionnaire also maintains sensitivity to age, disease, and phenotype, creating a holistic image of the ADL, covering a broad spectrum of daily activities from motor skills to cognitive function. Distinct from other ADL questionnaires, the body of this questionnaire is divided into three domains of "movement," "movement with cognition," and "cognition." These three domains are suitable for evaluating Hunter syndrome, in which both somatic and CNS manifestations are present.

Furthermore, since patients suffer from difficulties in their environment, it is important to assess not only impairment but also functionality [14]. As a result, this questionnaire scores the level of assistance the patient requires, resulting in a more precise evaluation of ADL. A sample size of 74 is the largest population of Japanese Hunter syndrome patients examined in an ADL study to date, in part due to its simplicity and suitability for evaluation of Hunter syndrome.

This study has evaluated the feasibility of this new questionnaire. Examination of the questionnaire data has demonstrated that (1) the questionnaire can distinguish patients with Hunter syndrome from age-matched controls, (2) the questionnaire can distinguish clinical phenotypes between "severe" and "attenuated," (3) early HSCT and ERT yield higher scores than late ERT and HSCT, respectively, and (4) early HSCT provides the highest score among treatment groups.

The scores of the age-matched controls were higher than those of patients with the severe phenotype of Hunter syndrome, and the difference in scores increased with age. This questionnaire has also shown that there is a significant difference between the severe and the attenuated phenotypes in all domains. However, due to limited respondents, no comparison between the two phenotypes could be made in the 0–5 years age-group. Our results are consistent with the current understanding that patients with severe phenotypes have more significant CNS involvement than patients with attenuated phenotypes who primarily have somatic involvement (Tables 1 and 2).

Table 3
ADL scores for severe forms of patients treated by ERT and HSCT.

		Subject age range (years)			
		0–5	5–10	10–15	>15
HSCT	*n*	1	6	4	7
	Movement	17	17.58 ± 2.31	13.5 ± 7.04	13.71 ± 5.82
	Movement cognition	7	8.5 ± 5.59	7.25 ± 5.44	10.86 ± 3.29
	Cognition	2	5.33 ± 1.37	3 ± 4.24	4 ± 3.29
Early ERT (under 8 years)	*n*	3	3	9	1
	Movement	15.33 ± 2.51	12.33 ± 2.31	10.33 ± 4.41	15
	Movement cognition	6.33 ± 2.52	6.66 ± 3.21	5.78 ± 3.77	8
	Cognition	4.66 ± 2.52	2.66 ± 2.88	3 ± 2.40	6
Late ERT (over 8 years)	*n*	–	–	2	11
	Movement	–	–	3.5 ± 4.95	3.77 ± 4.84
	Movement with cognition	–	–	2 ± 2.83	3.36 ± 3.61
	Cognition	–	–	2.5 ± 3.54	2.68 ± 3.35
t-test between HSCT and early ERT	Movement	–	NS	NS	–
	Movement with cognition	–	NS	NS	–
	Cognition	–	NS	NS	–
t-test between HSCT and late ERT	Movement	–	–	–	0.03
	Movement with cognition	–	–	–	0.03
	Cognition	–	–	–	NS

NS: no statistical significance at $p < 0.05$; HSCT: hematopoietic stem cell transplantation; ERT: enzyme replacement therapy.

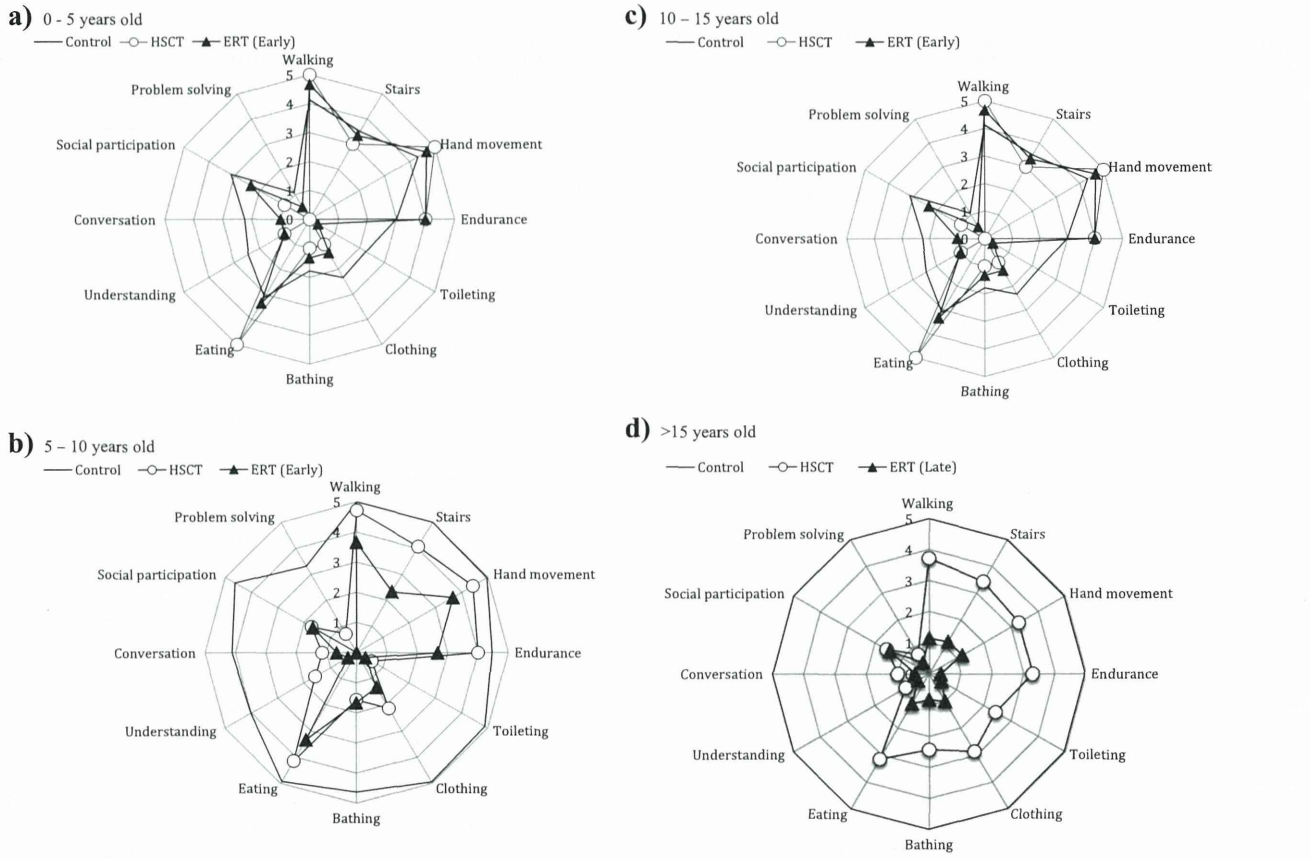

Fig. 4. Profile of subcategory scores for patients by treatment and age-group. (a) 0 years–5 years: control ($n = 46$); early ERT ($n = 3$); severe ($n = 4$). (b) 5 years–10 years: control ($n = 54$); early ERT ($n = 3$); HSCT ($n = 7$); severe ($n = 10$); attenuated ($n = 4$). (c) 10 years–15 years: control ($n = 25$); early ERT ($n = 9$); HSCT ($n = 4$); severe ($n = 15$); attenuated ($n = 8$). Late ERT ($n = 2$) is not shown due to low number of patients. (d) >15 years: control ($n = 13$); late ERT ($n = 11$); HSCT ($n = 7$); severe ($n = 21$); attenuated ($n = 13$). Early ERT ($n = 1$) is not shown due to low number of patients.

Although patients with attenuated phenotypes are characterized as showing perseveration of cognitive ability, the two older patients in this phenotype, aged 40 and 47 years, had low cognitive scores. This declining cognitive ability may be due to the severe hearing and visual impairment in these two patients, rather than a direct reduction in cognitive function. A larger cohort of older patients will be needed to determine whether a decline in cognitive score is feature of patients with attenuated Hunter syndrome.

An essential use of ADL questionnaires is to assess therapeutic efficacy and how certain treatments can improve the status of the patient's ADL. In this study, treatment groups were HSCT, early ERT, and late ERT. Several treated patients had been diagnosed as attenuated phenotype. Only severe phenotype patients were included in the treatment analysis because the attenuated phenotype patients would have likely scored high with or without treatment. HSCT patients could be divided into early and late treatment groups, but as patients in the two sub groups spanned similar age ranges, they were pooled to enable better statistical analysis. For ERT, it has become more common to begin treatment at an early age and consequently early and later ERT-treated patients span different age-groups and consequently can be analyzed separately with little loss of statistical power. The only groups for which statistically significant differences could be obtain was for patients older than 15 years of age showing better scores in HSCT-treated patients than late ERT-treated patients in "movement" and "movement with cognition" (Table 3). However, the oldest HSCT-treated patient in this study was 18 years old while the mean age of the late ERT group was over 20 years old. Nevertheless, all of the early HSCT-treated patients score in the top half of all treated patients and longer-term follow-up studies on the progress of these patients appear likely to show better outcomes for early HSCT. Although ADL scores of patients treated with HSCT were not statistically different from those treated with early ERT, average scores were higher for the HSCT-treated patients for each domain and each age-group (Table 3). While "movement" and "movement with cognition" scores appear to be stable across age-groups for HSCT-treated patients, cognition scores remain low for HSCT-treated patients. If patients could be diagnosed at birth through newborn screening, earlier treatment may be possible to help overcome reduced cognitive development in Hunter syndrome patients.

Our recent study on the blood HS level in patients with Hunter syndrome [20] showed (1) a significant increase in untreated patients with a severe form compared with the age-matched control group, (2) a significant increase in patients with a severe form compared to those with an attenuated form, (3) a significant decrease in the ERT- or HSCT-treated group compared with that in the untreated group, and (4) further reduction in patients treated by HSCT compared with patients treated by ERT. These results using HS as a biomarker are consistent with the better outcomes shown for HSCT patients in this current study. In future studies it will be of great interest to explore whether differences in blood HS levels are associated with ADL in patients with Hunter syndrome with or without treatment (or type of treatment).

In addition to the ADL, we also investigated early signs and symptoms of patients with Hunter syndrome since early diagnosis and early treatment are critical to improve quality of life. It is noteworthy that excessive growth, hernia, prominent Mongolian spot, or thick bone is observed during early stages of Hunter syndrome and that

recognition of these early signs and symptoms leads to early diagnosis. Excessive growth at a young age is also a feature of some other types of MPS [21,22]. During the first 4 years of life, the mean height for Japanese patients with Hunter syndrome is higher than that of an age-matched control population [22]. Clinical severity of CNS involvement in patients does not correlate with severity of growth impairment [21,22]. The etiology of the initial rapid growth followed by impairment of growth is not fully understood. Stimulation of the endocrine system for growth and other factors at an early stage could contribute to the early rapid growth. Further study of hormonal effect is needed to understand the mechanism of this early rapid growth.

In conclusion, we have determined the reliability of this questionnaire to show that it can distinguish the control subjects from the patients with Hunter syndrome and patients with severe phenotypes from attenuated phenotypes. We have also compared therapeutic efficacy, confirming the benefit of early treatment. HSCT provides a favorable consequence compared with ERT, although a significant difference between HSCT and early ERT remains to be supported. Moreover, future ADL evaluation of other MPS types or lysosomal storage disorders can be determined by using this questionnaire.

Compliance with ethics

The study was approved by the Institutional Review Boards (IRB) at Gifu University, at Saint Louis University, and at Nemours/Alfred I. duPont Hospital for Children.

Conflict of interest

All the authors contributed to the original article and have no conflict of interest with any other party.

Julian Tanjuakio, Yasuyuki Suzuki, Pravin Patel, Francyne Kubaski, Eriko Yasuda,

Akemi Tanaka, Hiromasa Yabe, Robert W. Mason, Adriana M. Montaño, Kenji E. Orii, Toshiyuki Fukao, Tadao Orii, and Shunji Tomatsu declare that they have no conflict of interests.

Contributions to the project:

Julian Tanjuakio contributed to the planning, data analysis, and reporting of the work described.

Yasuyuki Suzuki is a principal investigator for this project and has contributed to the concept, treatment of patients, planning of the project, informed consent, analysis of data, and reporting of the work described. He and his team conducted the project and followed up with the patients.

Pravin Patel has contributed to the planning, data analysis, and reporting of the work described.

Francyne Kubaski has contributed to the planning, data analysis, and reporting of the work described.

Eriko Yasuda has contributed to the planning, data analysis, and reporting of the work described.

Akemi Tanaka has contributed to the treatment of patients, data analysis, and reporting of the work described.

Hiromasa Yabe has contributed to the treatment of patients, data analysis, and reporting of the work described.

Robert W. Mason has contributed to the data analysis and reporting of the work described.

Adriana M. Montaño has contributed to the planning, data analysis, and reporting of the work described.

Kenji E. Orii has contributed to the treatment of patients and the planning, data analysis, and reporting of the work described.

Koji O. Orii has contributed to the data gathering, data analysis, and reporting of the work described.

Toshiyuki Fukao has contributed to the treatment of patients and the planning, data analysis, and reporting of the work described. He and his team conducted the project with Y. Suzuki.

Tadao Orii has contributed to the planning, data analysis, and reporting of the work described.

Shunji Tomatsu is a Principal Investigator for this project and has contributed to the concept and planning of the project, analysis of data, and reporting of the work described. He and his team conducted the project with Dr. Suzuki.

Acknowledgment

This work was supported by grants from the Austrian MPS Society and International Morquio Organization (Carol Ann Foundation). This work was also supported by Japanese MPS Family Society. Y.S. and K.E.O. were supported by the Ministry of Health, Labor and Welfare of Japan. R.W.M. and S.T. were supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of NIH under grant number P20GM103464. S.T. and A.M were supported by the National Institutes of Health grant R01HD065767. F.K. was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico from Brazil (CNPq). The content of the article is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health or other sponsors. Editorial assistance to the manuscript was provided by Michelle Stofa at Nemours/Alfred I. duPont Hospital for Children.

Appendix A. Supplementary

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ymgme.2014.11.002.

References

[1] R. Martin, M. Beck, C. Eng, et al., Recognition and diagnosis of mucopolysaccharidosis II (Hunter syndrome), Pediatrics 121 (2) (2008) e377–e386.
[2] T. Ochiai, K. Ito, T. Okada, M. Chin, H. Shichino, H. Mugishima, Significance of extensive Mongolian spots in Hunter's syndrome, Br. J. Dermatol. 148 (6) (2003) 1173–1178.
[3] I.D. Young, P.S. Harper, The natural history of the severe form of Hunter's syndrome: a study based on 52 cases, Dev. Med. Child Neurol. 25 (4) (1983) 481–489.
[4] I. Kuratsubo, Y. Suzuki, K.O. Orii, T. Kato, T. Orii, N. Kondo, Psychological status of patients with mucopolysaccharidosis type II and their parents, Pediatr. Int. 51 (1) (2009) 41–47.
[5] T. Kato, Z. Kato, I. Kuratsubo, et al., Evaluation of ADL in patients with Hunter disease using FIM score, Brain Dev. 29 (5) (2007) 298–305.
[6] P. Patel, Y. Suzuki, A. Tanaka, et al., Impact of enzyme replacement therapy and hematopoietic stem cell therapy on growth in patients with Hunter syndrome, Mol. Genet. Metab. Rep. 1 (2014) 184–196.
[7] A. Tanaka, T. Okuyama, Y. Suzuki, et al., Long-term efficacy of hematopoietic stem cell transplantation on brain involvement in patients with mucopolysaccharidosis type II: a nationwide survey in Japan, Mol. Genet. Metab. 107 (3) (2012) 513–520.
[8] S. Tomatsu, T. Fujii, M. Fukushi, et al., Newborn screening and diagnosis of mucopolysaccharidoses, Mol. Genet. Metab. 110 (1–2) (2013) 42–53.
[9] N.J. Mendelsohn, P. Harmatz, O. Bodamer, et al., Importance of surgical history in diagnosing mucopolysaccharidosis type II (Hunter syndrome): data from the Hunter Outcome Survey, Genet. Med. 12 (12) (2010) 816–822.
[10] J.E. Wraith, M. Scarpa, M. Beck, et al., Mucopolysaccharidosis type II (Hunter syndrome): a clinical review and recommendations for treatment in the era of enzyme replacement therapy, Eur. J. Pediatr. 167 (3) (2008) 267–277.
[11] J. Muenzer, J.C. Lamsa, A. Garcia, J. Dacosta, J. Garcia, D.A. Treco, Enzyme replacement therapy in mucopolysaccharidosis type II (Hunter syndrome): a preliminary report, Acta Paediatr. Suppl. 91 (439) (2002) 98–99.
[12] J. Marucha, A. Tylki-szymańska, J. Jakóbkiewicz-banecka, et al., 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 (9) (2011) 2257–2262.
[13] M. Richard, A. Arfi, J. Seguin, C. Gandolphe, D. Scherman, Widespread biochemical correction of murine mucopolysaccharidosis type VII pathology by liver hydrodynamic plasmid delivery, Gene Ther. 16 (6) (2009) 746–756.
[14] M.H. Gelb, F. Turecek, C.R. Scott, N.A. Chamoles, Direct multiplex assay of enzymes in dried blood spots by tandem mass spectrometry for the newborn screening of lysosomal storage disorders, J. Inherit. Metab. Dis. 29 (2–3) (2006) 397–404.
[15] E.M. Cotter, L.D. Burgio, A.B. Stevens, D.L. Roth, L.N. Gitlin, Correspondence of the functional independence measure (FIM) self-care subscale with real-time

observations of dementia patients' ADL performance in the home, Clin. Rehabil. 16 (1) (2002) 36–45.
[16] N.R. Guarany, I.V. Schwartz, F.C. Guarany, R. Giugliani, Functional capacity evaluation of patients with mucopolysaccharidosis, J. Pediatr. Rehabil. Med. 5 (1) (2012) 37–46.
[17] I. Wiklund, M. Raluy-callado, D.E. Stull, Y. Jangelind, D.A. Whiteman, W.H. Chen, The Hunter syndrome-functional outcomes for clinical understanding scale (HS-FOCUS) questionnaire: evaluation of measurement properties, Qual. Life Res. 22 (4) (2013) 875–884.
[18] I. Wiklund, M. Raluy-callado, W.H. Chen, J. Muenzer, J. Fang, D. Whiteman, The Hunter Syndrome-Functional Outcomes for Clinical Understanding Scale (HS-FOCUS) Questionnaire: item reduction and further validation, Qual. Life Res. 23 (9) (2014) 2457–2462.
[19] J. Muenzer, Early initiation of enzyme replacement therapy for the mucopolysaccharidoses, Mol. Genet. Metab. 111 (2) (2014) 63–72.
[20] T. Shimada, J. Kelly, W.A. LaMarr, N. van Vlies, E. Yasuda, R.W. Mason, W. Mackenzie, F. Kubaski, R. Giugliani, Y. Chinen, S. Yamaguchi, Y. Suzuki, K.E. Orii, T. Fukao, T. Orii, S. Tomatsu, Novel heparan sulfate assay by using automated high-throughput mass spectrometry: application to monitoring and screening for mucopolysaccharidoses, Mol. Genet. Metab. (Jul 21 2014), http://dx.doi.org/10.1016/j.ymgme.2014.07.008 (pii: S1096-7192(14)00212-1, [Epub ahead of print]).
[21] S. Tomatsu, A. Montano, H. Oikawa, R. Guigliani, P. Harmatz, M. Smith, T. Orii, Impairment of body growth in mucopolysaccharidoses, Handbook of Growth and Growth Monitoring in Health and Disease, Vol. 1, Springer New York, New York, NY, 2012, pp. 2091–2117.
[22] P. Patel, Y. Suzuki, M. Maeda, et al., Growth charts for patients with Hunter syndrome, Mol. Genet. Metab. Rep. 1 (2014) 5–18.

Molecular Genetics and Metabolism Reports 1 (2014) 184–196

Contents lists available at ScienceDirect

Molecular Genetics and Metabolism Reports

journal homepage: http://www.journals.elsevier.com/molecular-genetics-and-metabolism-reports/

Impact of enzyme replacement therapy and hematopoietic stem cell therapy on growth in patients with Hunter syndrome

Pravin Patel [a,1], Yasuyuki Suzuki [b,*,1], Akemi Tanaka [c], Hiromasa Yabe [d], Shunichi Kato [d], Tsutomu Shimada [a], Robert W. Mason [a], Kenji E. Orii [e], Toshiyuki Fukao [f], Tadao Orii [f], Shunji Tomatsu [a,f,**]

[a] Nemours/Alfred I. duPont Hospital for Children, Wilmington, DE, USA
[b] Medical Education Development Center, Gifu University, Japan
[c] Department of Pediatrics, Osaka City University Graduate School of Medicine, Osaka, Japan
[d] Department of Cell Transplantation & Regenerative Medicine, Tokai University School of Medicine, Isehara, Japan
[e] Division of Neonatal Intensive Care Unit, Gifu University Hospital, Japan
[f] Department of Pediatrics, Gifu University, School of Medicine, Gifu, Japan

ARTICLE INFO

Article history:
Received 1 April 2014
Accepted 1 April 2014
Available online xxxx

Keywords:
Hunter syndrome
Growth impact
Hematopoietic stem cell therapy
Enzyme replacement therapy
Height

ABSTRACT

Patients with Hunter syndrome (mucopolysaccharidosis II) present with skeletal dysplasia including short stature as well as CNS and visceral organ involvement. A previous study on Hunter syndrome indicated an impact on brain and heart involvement after hematopoietic stem cell therapy (HSCT) at an early stage but little impact after enzyme replacement therapy (ERT) (Tanaka et al. 2012). Meanwhile, impact on growth in patients with Hunter syndrome treated with ERT and HSCT has not been compared until now. We recently developed baseline growth charts for untreated patients with Hunter syndrome to evaluate the natural history of growth of these patients compared to unaffected controls (Patel et al., 2014).

To assess impact of ERT and HSCT on growth, clinical data were obtained from 44 Japanese male patients with MPS II; 26 patients had been treated with ERT, 12 patients had been treated with HSCT, and 6 had been treated

Abbreviations: DS, dermatan sulfate; ECM, extracellular matrix; ERT, enzyme replacement therapy; GAG, glycosaminoglycan; HSCT, hematopoietic stem cell therapy; HS, heparan sulfate; I2S, iduronate 2-sulfatase; LSD, lysosomal storage disorder; MPS II, mucopolysaccharidosis II.

* Correspondence to: Y. Suzuki, Medical Education Development Center, Gifu University, Yanagido-1-1, Gifu 501-1194, Japan.
** Correspondence to: S. Tomatsu, Skeletal Dysplasia Lab, Department of Biomedical Research, Nemours/Alfred I. duPont Hospital for Children, 1600 Rockland Rd., Wilmington, DE 19899-0269, USA. Fax: +1 302 651 6888.
E-mail addresses: ysuz@gifu-u.ac.jp (Y. Suzuki), stomatsu@nemours.org, stomatsu@gifu-u.ac.jp (S. Tomatsu).

[1] The first two authors should be regarded as joint first authors.

http://dx.doi.org/10.1016/j.ymgmr.2014.04.001

with both ERT and HSCT. Height and weight were compared to untreated patients and unaffected controls from the previous study.
We demonstrated 1) that MPS II patients, who had been treated with either ERT or HSCT, had increased height and weight when compared to untreated patients, and 2) that HSCT and ERT were equally effective in restoring growth of MPS II patients.
In conclusion, HSCT should be considered as one of the primary therapeutic options for early stage treatment of MPS II, as HSCT has also been reported to have a positive effect on brain and heart valve development (Tanaka et al. 2012).

1. Introduction

Mucopolysaccharidosis II (MPS II, Hunter syndrome; OMIM #309900) is a lysosomal storage disorder (LSD) caused by a mutation in the X-linked gene IDS. This results in deficiency of the lysosomal enzyme, iduronate 2-sulfatase (I2S), in the metabolic pathway that leads to degradation of the glycosaminoglycans (GAGs), dermatan sulfate (DS) and heparan sulfate (HS) [1]. This enzyme deficiency blocks the stepwise degradation of DS and HS, resulting in the accumulation of DS and HS in lysosomes and extracellular matrix (ECM) of a wide range of tissues. Although the primary result of enzymatic deficiency is accumulation of GAGs and secondary substrates, the mechanism causing the pathogenesis of the disease still remains unknown [2]. MPS II has a prevalence rate between 1:100,000 and 1:170,000 male births [3,4], and is the most prevalent form of MPS disorders in Asian countries where it accounts for around 50% of all MPS cases diagnosed [5].

Patients with MPS II have a wide range of symptoms caused by the disease that affect multiple different organ systems. The severe phenotype is more than twice as prevalent as the attenuated form of the disease, and is characterized by profound CNS involvement and is usually fatal in early childhood if not treated [6]. Patients with the attenuated phenotype may survive into adulthood without CNS involvement [1,7].

Inguinal and/or umbilical hernia, course facial features, otitis and nasal obstruction along with recurrent upper respiratory tract infections are some of the early diagnostic cues in MPS II [8]. Extensive and aberrant Mongolian spot is also a characteristic finding of Hunter syndrome in Japan [9]. The skeletal abnormalities in MPS II are similar regardless of clinical phenotype, and are common among other types of MPS disorders. The skeletal abnormalities are characterized in general as a thickening of the long bones with irregular ossification centers [1].

The major causes of morbidity and mortality in patients with MPS II are due to abnormal heart development; 82% of patients have cardiovascular signs and symptoms [1]. Detrimental CNS involvement in MPS II manifests most often as progressive cognitive degeneration, although individuals with the attenuated form of the disease have minimal CNS involvement. Patients may be able to reach early developmental milestones; however, psychomotor delays usually occur during the late infantile period [1]. Surgical procedures to correct inguinal and/or umbilical hernia are often performed before the diagnosis of Hunter syndrome [10].

There are currently two major therapies for patients with MPS II; enzyme replacement therapy (ERT) and hematopoietic stem cell therapy (HSCT).

ERT has been used to treat several types of MPS disorders, and for MPS II a recombinant form of human I2S is used (idursulfase, Elaprase®, Shire Human Genetic Therapies, Inc., Lexington, MA, USA). Clinical trials have shown that ERT decreases urinary GAG levels and improves measures of pulmonary function, walking ability, and visceral organ function [11–14]. Several limitations for conventional ERT have been noted: 1) limited efficacy for hard connective tissues including bone and heart valves due to avascularity of these tissues, 2) difficulty in compliance due to required 4–5 hour intravenous infusions every week, and 3) the high cost of treatment. Moreover, the enzyme cannot pass through the blood–brain barrier, and conventional ERT will have no effect on the CNS aspects of the disease [1,2,15,16].

Results from the Hunter Outcome Survey (HOS) show that response to treatment is not associated with either clinical phenotype or age at initiation of treatment [11]. MPS II patients treated with ERT continued to grow; however, their growth was not directly compared to untreated patients [11,17].

HSCT has been shown to be effective in the treatment of several MPS diseases and other LSDs. HSCT has been indicated for MPS II as part of standard care in Japan, leading to the fact that over 50 patients were treated by HSCT until now. The efficacy of HSCT on visceral organs was clear and similar to that of ERT. Tanaka et al. demonstrated that HSCT is effective in relieving both brain (CNS involvement) and heart defects, when treatment is performed at an early stage, before signs of brain atrophy and heart valve issues [18]. However, a systematic description of the impact of HSCT on growth of patients with MPS II has not been reported.

Thus, there remains little knowledge on the relative effectiveness of the two therapies in treating the skeletal deformities and overall growth in patients with MPS II. We recently resolved one of the underlying limitations of such studies by establishing growth charts for untreated patients with Hunter syndrome [19].

In this report we explored the impact of the two therapeutic options on growth of patients with MPS II. We evaluated the therapeutic effect on growth between patients treated with ERT or HSCT, in comparison with untreated patients with MPS II and age-matched healthy controls. We also propose therapeutic options for MPS II considering improvement or attenuation of other signs and symptoms.

2. Materials and methods

2.1. Study subjects

Patients diagnosed with MPS II by enzyme assay were recruited at Gifu University to participate in this study by providing clinical history and growth data. A questionnaire was sent to local medical centers in Japan where patients with MPS II had received ERT and/or HSCT. Informed consent was obtained from the patients and/or their guardians by the attending physicians. The study was approved by the Institutional Review Boards (IRB) at Gifu University and at the Nemours/Alfred I. duPont Hospital for Children. Seventy-two patients treated with ERT and/or HSCT were enrolled in this study. 28 patients were excluded to focus on the 44 Japanese male patients who had started treatment at or before 8 years of age so that we could observe the impact on growth. Twenty-six of these patients were treated with ERT alone, and 12 were treated with HSCT alone. The remaining 6 patients were treated initially with ERT, and then later with HSCT. Their growth data was added to the respective treatment groups during the times when they were receiving treatment. There were 4 sibling cases included in our study.

2.2. Anthropometric measurements

Measurement of height was performed using the health-check system in Japan (infantile health-check at public healthcare centers in local government, health-check in schools and/or in hospitals). Anthropometric measurements were taken using a standard technique and included body length and weight. Until 3 years of age, the lengths of patients were measured in the supine position using a liberometer. After 3 years of age, height was taken in a standing position using a stadiometer, and was fairly objective, although height measurements might be affected due to structural abnormalities. Measurements were made an average of 3 times per patient.

2.3. Treatment

ERT was administered 1 mg/kg weekly. Patients who had received HSCT and had confirmed donor cell engraftment were chosen to participate in this study.

2.4. Statistical analysis

Data used for the construction of growth curves were age (years and months), height (cm), and weight (kg). Body mass index (BMI) was calculated from height and weight data by dividing weight by height squared (kg/m^2). Statistical analyses for mean and standard deviation were then performed on the data sets. Student's *t*-test was performed on the height, weight, and BMI data for patients according to therapy type to determine the statistical relationship between these two patient groups. These data were also

compared with the values in untreated patients with MPS II [19]. The standard control data was taken from established Japanese data sets for healthy male controls issued by the Japanese Ministry of Health, Labor, and Welfare. Control data from untreated subjects were obtained from a different study that utilized identical testing within the same laboratory. This study was based upon the data obtained from 111 Japanese male patients with MPS II. On average, height and weight measurements were obtained at 8 time points for each patient [19].

3. Results

3.1. Demographics

The mean age of symptom onset was 24 ± 20 months and the mean age at diagnosis was 37 ± 21 months. Mean age at start of ERT was 4.49 ± 2.35 years, while mean age at start of HSCT was 4.68 ± 1.63 years, suggesting that no significant difference in the age at start of therapy was observed. Of the 44 patients included in our study, 35 had the severe phenotype, and 9 had the attenuated phenotype. One patient diagnosed with the attenuated phenotype was treated with HSCT, while 7 were treated with ERT, and one was treated with ERT then HSCT. Eleven patients with the severe phenotype were treated with HSCT, while 19 were treated with ERT, and 5 were treated first with ERT and successive HSCT.

3.2. Heights

ERT-treated patient heights were not significantly different from untreated patient heights for children younger than 8 years of age, but older treated patients were significantly taller (Table 1, Fig. 1), suggesting that long-term observation is required to see an impact on growth.

Similarly, HSCT treated patients were not significantly different from untreated patients for children younger than 8 years of age but were significantly taller than untreated patients from 10 to 18 years of age (Table 1, Fig. 1). The children approaching maturity were 20 cm taller than the age-matched untreated patients.

Heights of treated or untreated patients were similar to normal controls for patients younger than 6 years of age (Fig. 1). After 6 years of age, growth of untreated patients is significantly slowed (19 and Fig. 1). While the growth rate of ERT treated patients is less than that of the controls it is clearly faster than that of untreated patients (Fig. 1).

In this study both treatments showed a similar effect in improving growth curves for MPS II (Table 1, Fig. 1).

Table 1
Height of patients with MPS II undergoing therapy.

	HSCT (cm)		*t*-Test to untreated group		ERT (cm)		*t*-Test to untreated group		*t*-Test between two treatment groups
Age (years)	n	Mean	SD	p	n	Mean	SD	p	p
9.5 months					1	72.80			
1 y					1	74.50			
1.5 y					4	84.25	3.96	0.83	
2 y	1	94.00			4	89.38	3.12	0.88	
3 y	1	97.00			5	100.40	4.84	0.22	
4 y	4	103.65	3.03	0.25	6	103.87	3.28	0.15	0.92
5 y	9	107.42	8.02	0.87	5	110.84	5.17	0.17	0.35
6 y	12	110.92	4.95	0.97	8	113.95	5.72	0.20	0.24
8 y	9	119.57	6.00	0.13	11	119.06	5.08	0.11	0.84
10 y	10	126.47	8.25	<0.01	7	129.10	5.43	<0.005	0.44
12 y	9	133.12	10.36	<0.005	7	133.01	9.93	<0.01	0.98
14 y	6	142.38	8.02	<0.005	1	138.00	–	–	
16 y	4	149.70	3.23	<0.005					
18 y	2	147.00	–	–					