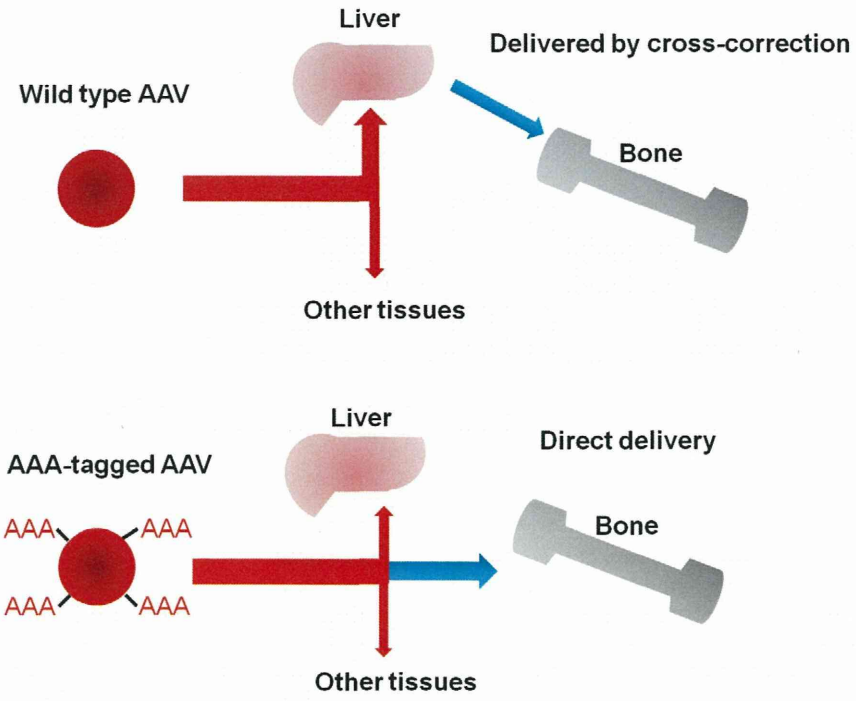

Fig. 9. Mechanism of multiple-AAA targeting system. Viral capsid in the right panel has multiple copies of D8 integrated into capsid proteins, showing the retargeting of gene vector to bone (hydroxyapatite in the mineral region) schematically.

urine and tissues, less pronounced facial dysmorphism, increased body weight and improvement in appendicular skeleton lengths, articular cartilage erosion, and cervical vertebral articular process widening, and mobility. Nevertheless, there was limited effect in cervical vertebrae, cervical vertebral fusion, and intervertebral disk degeneration [131]. These results showed that an early treatment with a long-term supraphysiological enzyme activity levels was not able to completely resolve all skeletal abnormalities.

2.4.4. MPS VII

Gene therapy reports for MPS VII have used plasmid [132] sleeping beauty transposon [117], gamma-retroviral [120,121,132,133], lentiviral [134], and AAV [135,136] vectors.

Favorable results for bone lesions were reported in MPS VII dogs by neonatal gene therapy using a gamma-retroviral vector [133]. Treated MPS VII dogs were followed for up to 11 years, which showed maintained therapeutic levels of enzyme activity associated with a reduction of GAG levels in urine and tissues. Treated MPS VII dogs showed a significant correction for most bone deformities and could walk throughout their lives, while untreated MPS VII dogs could not stand beyond 6 months and were dead by 2 years. Luxation of the coxofemoral joint and the patella, dysplasia of the acetabulum and supracondylar ridge, deep erosions of the distal femur, and synovial hyperplasia were reduced, and the quality of articular bone was improved in treated dogs [133]. Nevertheless, treated dogs continued to have osteophyte formation, cartilage abnormalities, calcification of the ventral epiphysis of the vertebral bodies, and intervertebral disk degeneration, resulting in an abnormal gait. Thus, neonatal gene therapy reduces some skeletal abnormalities in MPS VII dogs and dramatically improves their life span, but clinically-relevant abnormalities in bone remain due to the inability of GUSB to diffuse into spine tissues, suggesting that ERT will probably have similar limitations long-term [120,121,132,133].

Neonatal and adult gene therapy in MPS VII mice by the use of lentiviral vectors showed improvement in parameters of bone mass and architecture as well as biochemical and enzymatic correction [134]. However, growth plate chondrocytes were not responsive to treatment, as evidenced by the lack of improvement in vertebral and femoral bone length and growth plate height.

In summary, gene therapies for MPS animal models have shown a favorable safety profile with long-term expression periods over 10 years for the viral vectors, and promising results for non-viral vectors. Both vectors lead to substantial impact in bone lesion in animal models, when supraphysiological enzyme activity levels are maintained. However, spine deformities especially on large animal models remain challenging to correct, even with neonatal therapy and supraphysiological levels of the enzyme activity. The results for MPS IVA show the potential to treat the bone lesion by using a novel system of AAV vectors with a bone-targeting system. Further studies should shed light on the long-term evaluation of gene therapy with both viral and non-viral vectors, which will show a great potential for the treatment of bone disease in MPS.

2.5. Anti-inflammatory drugs

In patients with MPS, chronic osteoarthritis associated with skeletal dysplasia can happen in any major joints such as shoulder, wrist, hip, knee, and ankle. In the last decade, there have been several key reports showing that inflammatory responses exacerbate MPS symptoms. Accumulated GAGs (keratan sulfate; KS, chondroitin-6-sulfate; C6S) in bone, cartilage, and extracellular matrix (ECM) induce pro-inflammatory factors (e.g. TNF-α, RANTES, TIMP-1, and MIP-1α IL-1, -2, -5), that lead to cartilage degradation by degradative proteases (e.g. MMPs), and subsequently chronic osteoarthritis and spondyloepiphyseal dysplasia. Chondrocytes and ECM in patients with MPS are markedly vacuolated and tissues are affected with appearance of foam cells, macrophages, and T-cells (Fig. 10), suggesting that inflammation plays a key role of skeletal dysplasia [137].

To suppress metabolic inflammation caused by GAG accumulation, two treatments are available: one is to reduce the causative factor (reduce GAGs by ERT, gene therapy, SRT, HSCT etc.), while the other is to inhibit secondary inflammatory processes using anti-inflammatory (or immunosuppressive) agents. These anti-inflammatory agents have

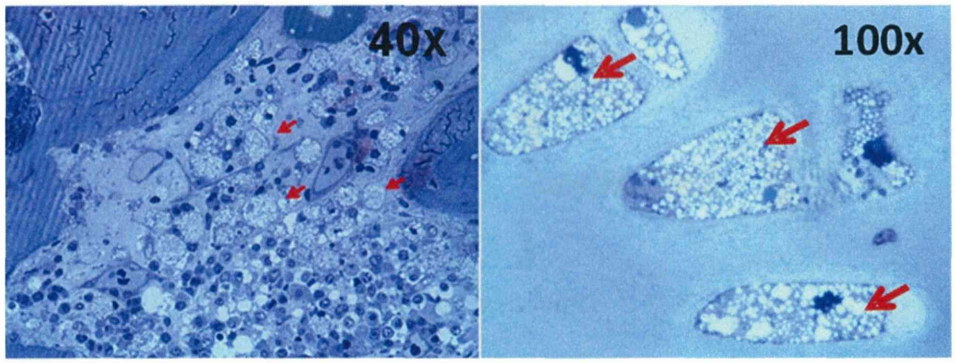

Fig. 10. Appearance of foam cells/macrophages/vacuolated cells in tissues in autopsied specimens from a 20-year-old male MPS IVA patient. Left; bone marrow in the vertebrae shows foam cells and vacuolated osteoblasts (40×), right; trachea shows ballooned vacuolated chondrocytes (100×). Stained with toluidine blue (0.5 μm; light microscopy).

distinct mechanisms of action, including inhibition of the action of cytokines, blocking cell–cell interactions, and depleting certain cell types. TNF-α is a dominant proinflammatory cytokine in the pathophysiology of MPS, and several biologic agents are approved to target this cytokine to treat autoimmune diseases such as rheumatoid arthritis (RA).

The effect of anti-TNF-α (infliximab) therapy was assessed in MPS VI rats. Early treatment in the presymptomatic period inhibited the elevation of TNF-α, RANKL and other inflammatory factors in the blood, articular chondrocytes and synovial fibroblasts [36]. The number of apoptotic articular chondrocytes was reduced, and there was no difference from healthy control rats. However, there was no impact on bone growth or mobility since stored GAGs still remained in chondrocytes of the growth plate. The efficacy of ERT alone and combined treatment using ERT and anti-TNF-α drug (specific monoclonal antibody against TNF- α: CNTO1081) was also tested [37]. Both treatments markedly reduced serum levels of TNF-α and RANKL, although only the combined treatment reduced TNF-α in the articular cartilage. Analysis of cultured articular chondrocytes showed that combination therapy restored collagen IIA1 expression and reduced expression of apoptotic markers. Only the combined therapy suppressed hyperplasia of synovial cells into underlying bone and clinical effects on other organs that are not accessible to the enzyme (e.g. cartilage) [37]. However, these therapies do have adverse effects.

Pentosan polysulfate (PPS) has potent anti-inflammatory effects and is an FDA-approved drug used for patients with interstitial cystitis and has also been used for thrombosis prophylaxis and to treat phlebitis in Europe for several decades. Successively, clinical trials of PPS in patients with knee osteoarthritis provided significant reduction in pain [138]. Schuchman et al. also reported that oral and subcutaneous administration of PPS reduced inflammation and improved skeletal pathology, bone mineral density, and mobility of joints in MPS VI rats [39,139, 140]. Ghosh et al. reported that PPS promotes proliferation, and chondrogenic differentiation of adult human bone marrow-derived mesenchymal precursor cells [139]. In our preliminary experiment, PPS suppressed GAG accumulation in fibroblasts of several types of MPS patients. Osteoarthritis is one of the primary concerns in patients with MPS. Progressive bone and joint disorders in MPS cause severe pain, resulting in disability of walking and poor ADL. Since PPS reduced pain in interstitial cystitis and osteoarthritis patients [138], anti-inflammatory effects of PPS could provide improvements of ADL and QOL in patients with MPS. PPS will provide suppression of inflammation, reduction of GAG accumulation, and/or promotion of chondrogenesis in chondrocytes of the patients with MPS (Fig. 11).

A clinical study and a trial of PPS for patients with MPS I and II started in 2014 and adverse effects and therapeutic efficacy are under investigation.

3. Conclusion

Resolution of bone and cartilage issues remains an unmet challenge for patients with MPS. Patients with MPS have severe progressive skeletal dysplasia that leads to significant morbidity and handicap with poor ADL. Management requires multidisciplinary approaches for the patient, particularly for those who have serious issues such as spinal cord compression, ambulatory problems, and restrictive and obstructive lung issues. A comprehensive assessment of individual patient at initial diagnosis is required, and continued follow-up by primary care clinicians. Supportive management, physiotherapy, and appreciation of possible complications can also improve the QOL of MPS patients and their families. Families of the patients should be offered tailor-made management including genetic counseling, choice of ERT, HSCT, gene therapy (if it becomes available), anti-inflammatory drugs, supportive therapies, physiotherapies and orthopedic interventions. Physicians who take care of MPS patients should be familiar with the most common complications, diagnosis of the disease, and locations of expert centers as well as available therapies. Metabolic and transplant doctors as well as genetic counselors should cooperatively examine the range of therapeutic options to provide the optimal outcome for individual patients. Hopefully, this will lead to earlier diagnosis for patients, resulting in better comprehensive therapy and avoidance of progression to irreversible damage. ERT, HSCT, gene therapy, and anti-inflammatory drug are therapies that could be offered before or after onset of the disease. Although the current treatments will not cure the disease, they provide the potential to rescue most patients from consequences of the disease and to

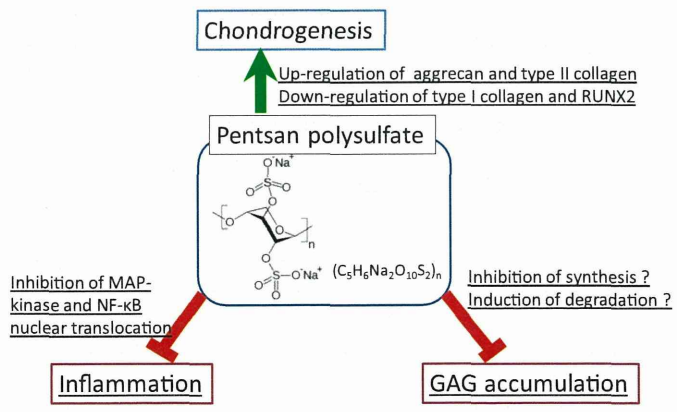

Fig. 11. Hypothesis for role of PPS on reduction of GAGs, suppression of inflammation, and promotion of chondrogenesis in chondrocytes RUNX2: runt-related transcription factor 2, MAP: mitogen-activated protein, NF-κB: nuclear factor-kappa B.

improve the QOL. It seems that therapy outcomes are better if treatment is started at an early stage, which should drive support for policies that advocate newborn screening for these diseases. Established systemic bone dysplasia remains a serious challenge, and robust, innovative approaches such as bone targeting should be considered. Longitudinal observation of MPS patients under current therapies provides more precise and valuable information regarding the appropriate assessment, including physical activity, supportive treatment, efficacy of therapy, and the clinical endpoints.

Conflict of interest

All the authors contributed to the Review Article and had no conflict of interest with any other party. Shunji Tomatsu, Carlos J. Alméciga-Díaz, Adriana M. Montaño, Hiromasa Yabe, Akemi Tanaka, Vu Chi Dung, Roberto Giugliani, Francyne Kubaski, Robert W. Mason, Eriko Yasuda, Kazuki Sawamoto, William Mackenzie, Yasuyuki Suzuki, Kenji E. Orii, Luis A. Barrera, William S. Sly, and Tadao Orii declare that they have no conflict of interests. Part of the content was presented at 13th International Symposium on Mucopolysaccharidoses and Related Diseases (Bahia, Brazil; August 13–17, 2014).

Contributions to the project

Shunji Tomatsu is a Principal Investigator for this review article and has contributed to the concept and planning of the article, collection of data, and reporting of the work described.

Carlos J. Alméciga-Díaz contributed to the planning of the article, collection of data on gene therapy, and reporting of the work described.

Adriana M. Montaño contributed to the planning of the article, collection of data on ERT and gene therapy, and reporting of the work described.

Hiromasa Yabe contributed to the planning of the article, collection of data on HSCT, and reporting of the work described.

Akemi Tanaka contributed to the planning of the article, collection of data on ERT and HSCT, and reporting of the work described.

Vu Chi Dung contributed to the planning of the article, collection of data on ERT and mouse pathology, X-ray pictures, and reporting of the work described.

Roberto Giugliani contributed to the planning of the article, collection of data on ERT and X-ray pictures, and reporting of the work described.

Francyne Kubaski contributed to the planning of the article, collection of data in HSCT, and reporting of the work described.

Robert W. Mason contributed to the planning of the article, collection of data, and reporting of the work described.

Eriko Yasuda contributed to the planning of the article, collection of data in pathology, and reporting of the work described.

Kazuki Sawamoto contributed to the planning of the article, collection of published data in ERT, gene therapy and HSCT, and reporting of the work described.

William Mackenzie contributed to the planning of the article, collection of data on surgical specimen, and reporting of the work described.

Yasuyuki Suzuki contributed to the planning of the article, collection of data on ERT and HSCT, and reporting of the work described.

Kenji E. Orii contributed to the planning of the article, collection of data on ERT and HSCT, and reporting of the work described.

Luis A. Barrera contributed to the planning of the article, collection of data on gene therapy, and reporting of the work described.

William S. Sly contributed to the planning of the article, collection of data on ERT, and reporting of the work described.

Tadao Orii is a Principal Investigator for this review article and has contributed to the concept of the manuscript, planning of the article, collection of data, and reporting of the work described.

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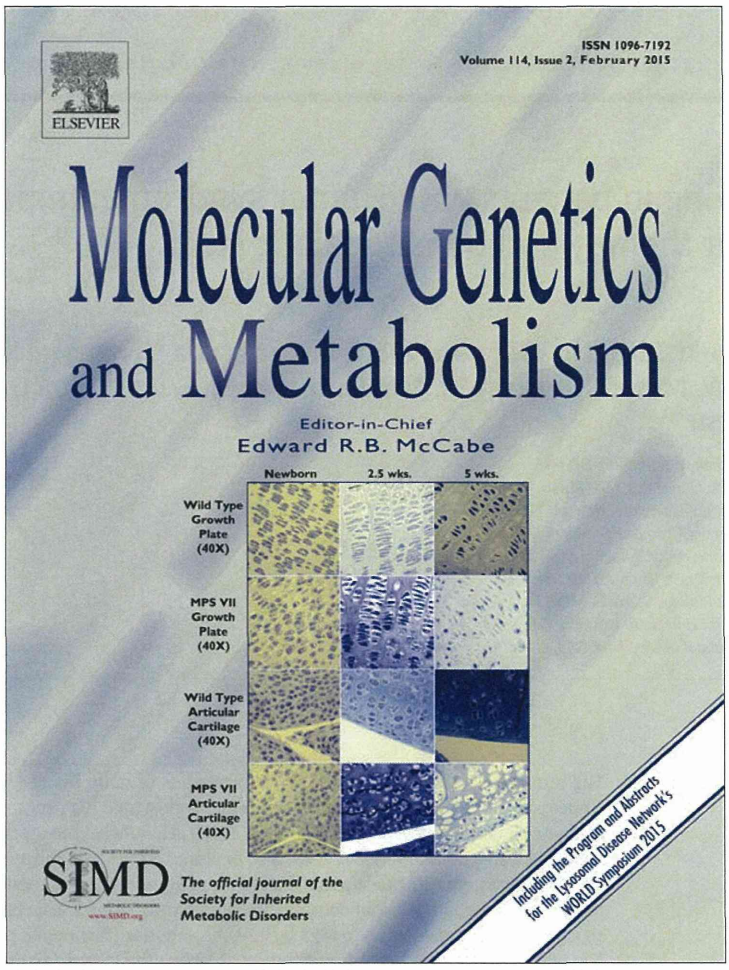

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Activities of daily living in patients with Hunter syndrome: Impact of enzyme replacement therapy and hematopoietic stem cell transplantation

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ABSTRACT

The aim of this study was to assess the activities of daily living (ADL) in patients with Hunter syndrome (mucopolysaccharidosis II; MPS II) using a newly designed ADL questionnaire. We applied the questionnaire to evaluate clinical phenotypes and therapeutic efficacies of enzyme replacement therapy (ERT) and hematopoietic stem cell transplantation (HSCT). We also explored early signs and symptoms to make early diagnosis feasible.
We devised a new ADL questionnaire with three domains: "movement," "movement with cognition," and "cognition." Each domain has four subcategories rated on a 5-point scale based on level of assistance. We also scored signs and symptoms unique to MPS by 12 subcategories (five points per category), providing 60 points in total. The questionnaire was first administered to 138 healthy Japanese controls (0.33–50 years), and successively, to 74 Japanese patients with Hunter syndrome (4–49 years). The patient cohort consisted of 51 severe and 23 attenuated phenotypes; 20 patients treated with HSCT, 23 patients treated early with ERT (≤8 years), 25 patients treated late with ERT (>8 years), and 4 untreated patients. Among 18 severe phenotypic patients treated by HSCT, 10 were designated as early HSCT (≤5 years), while 8 were designated as late HSCT (>5 years).
Scores from patients with severe phenotypes were lower than controls and attenuated phenotypes in all categories. Among patients with severe phenotypes, there was a trend that HSCT provides a higher ADL score than early ERT, and there was a significant difference in ADL scores between late ERT and HSCT groups. Early ERT and early HSCT provided a higher score than late ERT and late HSCT, respectively.
In conclusion, we have evaluated the feasibility of a new questionnaire in control population and patients with Hunter syndrome, leading to a novel evaluation method for clinical phenotypes and therapeutic efficacy. Early treatment with HSCT provides a better consequence in ADL of patients.

1. Introduction

Hunter syndrome (mucopolysaccharidosis II; MPS II) is an X-linked recessive lysosomal storage disorder caused by a deficiency of iduronate-2-sulfatase (IDS). IDS is required for the degradation of the glycosaminoglycans (GAGs), dermatan sulfate (DS), and heparan sulfate (HS). Deficiency of this enzyme results in the accumulation of GAG in most cell types and tissues, leading to the progressive damage

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to the bone, cartilage, upper and lower respiratory tract, lung, heart, and brain. Clinical manifestations include coarse facial feature, umbilical hernia, inguinal hernia, marked Mongolian spots, obstructive airway disease, recurrent nose and ear infections, and skeletal deformities [1]. Patients commonly show initial excessive growth in the first few years followed by growth retardation, umbilical hernia, inguinal hernia, and thick bones [1]. In Asian populations, an early sign is appearance of a prominent Mongolian spot [2]. Ultrastructural findings of Mongolian spots suggest that the hyperpigmentation is a long-lasting symptom. Detection of Mongolian spots may lead to early diagnosis in patients with a mild form of Hunter syndrome [2].

Clinical cases of Hunter syndrome are ranked on a continuum from attenuated phenotypes to severe phenotypes. Severe and attenuated phenotypes are differentiated by the presence or absence of cognitive impairment. The severe phenotype of Hunter syndrome, which is twice as prevalent as the attenuated form, is characterized by significant CNS involvement such as mental retardation and loss of cognitive function [3–7]. Untreated patients usually do not survive past their second decade of life [1,3]. Patients with attenuated phenotypes are mainly characterized by somatic involvement without CNS involvement. Surgical operations of umbilical/inguinal hernia repair, tonsillectomy, adenoidectomy, and ear tubes are common in Hunter syndrome [1,8,9]. Risk factors of mortality in patients with Hunter syndrome include severe upper airway constriction and abnormal heart development causing left and right ventricular hypertrophy, heart valvular involvement, and heart failure [1,9].

Although there is no cure for Hunter syndrome, current treatments include enzyme replacement therapy (ERT) [10,11] and hematopoietic stem cell transplantation (HSCT) [6,7], to reduce the accumulation of GAG. Studies have shown that ERT can reduce GAG levels in both urine and plasma; improve lung, heart, and other visceral organ function; and prolong a patient's life. However, limitations of ERT result from (1) its high cost, (2) inability of the enzyme to cross the blood–brain barrier, (3) the need for weekly infusion for 4–5 h at a medical facility, and (4) limited impact on CNS, avascular cartilage, and the skeletal system [9]. HSCT has proved to be similar to or better than ERT in terms of reduction of GAG levels and organ function [6,7]. Previous studies have shown beneficial therapeutic effects of ERT and HSCT on growth in patients [6,7], but a comparison of the relative benefits of ERT and HSCT on activities of daily living (ADL) has not been reported previously. Gene therapy for Hunter syndrome and substrate reduction therapy (SRT) for MPS in general are potential alternative treatments that are currently in clinical trials [12,13]. Regardless of treatment mode, early treatment yields the most significant impact in ameliorating disease symptoms [5–7]. Newborn screening for Hunter syndrome is now under development [8,14].

To assess the ADL and other clinical end points, patients and their families with Hunter syndrome have taken several tests and completed a range of questionnaires. The Functional Independence Measure (FIM) is widely used to evaluate ADL in patients with a range of disabilities [15], including Hunter syndrome [4,5,16]. Originally designed for patients with neurological disorders such as dementia and multiple sclerosis, the FIM questionnaire tests the patients on motor and cognitive function. The need for a trained professional to administer the questionnaire makes the assessment inconvenient and time consuming for the family and patients. Other types of questionnaires used to evaluate ADL in Hunter syndrome patients include the Hunter syndrome-functional outcomes for clinical understanding scale (HS-FOCUS) [17,19] and the Pediatric evaluation of Disability inventory (PEDI) for general child health [16]. To date, only a few studies have evaluated the clinical status of Hunter syndrome [9,14–18], and the effect of ERT and HSCT on ADL in patients with Hunter syndrome has not been reported yet.

In this report, we have created a new, simple ADL questionnaire that can be easily completed by patients or their parents. We have evaluated its value using normal control subjects, showing increased ADL in older children. Successively, we showed that responses to the questionnaire can separate MPS II patients from age-matched healthy controls and also distinguish patients with severe and attenuated phenotypes. We have applied this questionnaire to compare the impact of ERT and HSCT on ADL.

2. Subjects and methods

2.1. Study subjects

All control and Hunter syndrome patients were of Japanese ethnicity and were enrolled, with informed consent at local Japanese hospitals. Normal control subjects ($n = 138$; 75 males, 63 females; age range 0.33–50 years old; average age, 8.34 ± 7.75 years) and patients with Hunter syndrome ($n = 74$; age range, 3–47 years old; average age 17.27 ± 8.69 years) were enrolled. Fifty-one patients were diagnosed with severe phenotypes (diagnosed mean age; 3.09 ± 1.57 years), and 23 patients were diagnosed with attenuated phenotypes (diagnosed mean age; 4.4 ± 2.03 years). Twenty-three patients were treated with early ERT alone (treatment before the age of 8 years), 25 patients were treated with late ERT alone (treatment after the age of 8 years), 12 patients were treated with HSCT alone, 8 patients were treated with both ERT and HSCT, and 4 patients were untreated. The average age at which patients started treatment using ERT was 5.2 ± 2.2 years for early-stage group and 17.8 ± 6.6 years for the late-stage group. The average age at which patients started treatment using HSCT was 3.6 ± 1.1 years for early-stage group (before 5 years of age) and 5.9 ± 1.6 years for the late-stage group (after 5 years of age).

Average age of treatment was 4.8 ± 1.6 years for HSCT-treated patients. Average time since HSCT had been performed was 10.6 ± 9.49 years, and average since ERT was 6.0 ± 2.0 years.

2.2. Questionnaire

The questionnaire (12 items) used (see Supplement Fig. 1) consisted of three domains ("movement," "movement with cognition," and "cognition"); each with four subcategories scored from 0 to 5 (0 being the inability to perform task without maximum assistance and 5 being the ability to perform a task without any assistance). "Movement" comprised basic motor skills needed for normal daily function with the subcategories: (1) walking, (2) movement on stairs, (3) grasping/finger movement, and (4) endurance in a 6-min walk (6 MWT). "Movement with cognition," daily activities that required some level of cognitive awareness, comprised (1) toileting, (2) changing clothes, (3) bathing, and (4) eating. For "cognition," subcategories included (1) understanding of everyday conversation, (2) conversation and speaking with others, (3) social participation, and (4) problem solving. The maximum possible score that could be obtained was 20 per domain with a total of 60 points.

In a second section of the questionnaire, twelve other symptoms specific to MPS were assessed including: work/study, behavioral problems, sleep, pain, joint flexion, respiratory status, infection, vision, hearing, skin, hair, and appetite. These were all scored on 0–5 scales.

In a third section, patients and their parents were asked about growth (birth, 1.5 years, and 3 years), initial and early signs and symptoms of Hunter syndrome, and surgical history (Fig. 1).

Questionnaires were mailed to families, completed directly by the patient and/or the patient's parent/guardian, and then returned to the study group.

2.3. Statistical analysis

Means and standard deviations of the total score and each domain were calculated by age-groups 0–5, 5–10, 10–15, and >15 years of age. Student's *t*-test was used to compare the means of (1) the patients with Hunter syndrome to age-matched normal controls, (2) patients with the severe phenotypes to patients with attenuated phenotypes, (3) ERT patients to HSCT patients, (4) early ERT or HSCT patients to late ERT or HSCT. Most patients were classified as severe, and therefore, there was

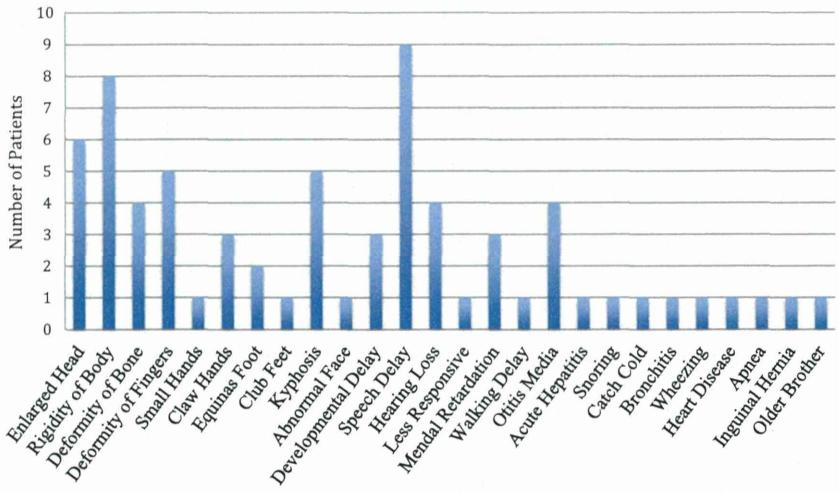

Fig. 1. Initial signs and symptoms of patients with Hunter syndrome ($n = 73$).

insufficient data to compare treatments for patients with an attenuated phenotype.

Statistical analyses were performed using Student t-test with SPSS v.16. The significance level was set at $p < 0.05$.

3. Results

3.1. Early signs and symptoms

The reported initial signs and symptoms included bone deformity, failure of thrive, kyphosis, scoliosis, abnormal gait, difficulty of joint movement, herniation, knee problem, corneal clouding, chronic ear infections, short neck, and heart murmur (Fig. 1). At early stages of the disease, patients were frequently found to have inguinal hernia, umbilical hernia, prominent Mongolian spot, and thick bones (Table 1). Early signs and symptoms, including extensive Mongolian spots, did not distinguish severe and attenuated phenotypes. Inguinal hernia repair (53%), ear tub (49%), and adenoidectomy (32%) were the most frequent surgical procedures in both phenotypes (Table 1).

Table 1
Growth pattern, early signs and symptoms, and surgical operations.

		Attenuated	Severe
N		23	51
Age (years)		20.22 ± 10.45	13.67 ± 6.07
Height above 97th percentile	Birth	5%	4%
	1.5 years	17%	38%
	3 years	18%	13%
Weight above 97th percentile	Birth	5%	10%
	1.5 years	44%	63%
	3 years	29%	63%
Height or weight above 97th percentile	Birth	10%	10%
	1.5 years	50%	65%
	3 years	35%	63%
Height and weight above 97th percentile	Birth	0%	6%
	1.5 years	33%	35%
	3 years	18%	13%
Early symptoms	Umbilical hernia	26%	16%
	Inguinal hernia	17%	26%
	Mongolian spot	91%	61%
	Thick bone	57%	54%
Operations	Umbilical hernia	26%	14%
	Inguinal hernia	61%	49%
	Tonsillectomy	17%	20%
	Adenoidectomy	26%	35%
	Ear tube	48%	49%
	Shunt	0%	0%
	Cervical fusion	4%	2%
	Hip surgery	0%	0%
	Heart valve	0%	2%

Excessive growth at an early age was noticed in Hunter syndrome patients. Birth weights and lengths of patients were near normal, but by aged 1.5 and 3 years, 30% and 15%, respectively, had both weight and height above the 97th percentile, confirming excessive growth in Hunter syndrome patients (Table 1). More than 50% of patients had weights above the 97th percentile of the normal population at both ages.

3.2. ADL scores in control subjects

3.2.1. Total scores

In control subjects, all scores increased with age (Fig. 2). The total score reached a plateau at around 10 years of age, and all control subjects older than 15 years of age obtained a maximum score (Fig. 2a).

"Movement" scores reached a plateau at around 7 years of age (Fig. 2b), and "movement with cognition" scores reached a plateau at about 8 years of age (Fig. 2c). "Cognition" scores developed more slowly, reaching a plateau at 11 years of age (Fig. 2d).

3.3. Comparison of ADL scores between controls and patients with a severe or attenuated phenotype

Total scores for patients with a severe phenotype ranged from 0 to 43 (Fig. 3a). Unlike control subjects, scores decreased with age for these patients. Cognitive function showed the greatest decline with age in these patients. Twenty-five of 35 patients more than 10 years old had a cognition score of 5 or less. The mean score was significantly lower than that of the age-matched controls across all domains and age-groups for these severe phenotypic patients (Table 2).

In patients with an attenuated phenotype, total scores increased with age, similar to that of control subjects (Fig. 3a). These patients had almost perfect scores between 15 and 30 years of age, but the two older patients had lower scores than controls. Patients with attenuated phenotypes had significantly higher scores than patients with severe phenotypes, in all categories and across all age-groups (except for patients aged 0–5 in the Movement domain) (Table 2, Supplemental Fig. 2).

Among other symptoms related to MPS, patients with a severe phenotype had lowest scores in behavioral problems and joint flexion and also had low scores in hair and skin quality and work/study habits (Supplemental Fig. 3). Patients with an attenuated phenotype had better scores than severe phenotype patients for all symptoms apart from joint flexion (Supplemental Fig. 3).