Table 4 Clinical characteristics (subjects stratified by the median-normal value of serum FT_4). Data are expressed as mean±S.D.

	$FT_4 \geq 1.10$					$FT_4 < 1.10$				
	CeH	Tx	PH	C	*P* value[a]	CeH	Tx	PH	C	*P* value[a]
Age (y)	46.6±16.9[‡]	55.9±14.4	51.9±18.9	65.7±11.0[‡]	0.006	56.5±20.6	58.6±17.8	56.6±13.9	58.8±16.9	0.07
Gender (male/female)	3/9	4/10	1/18	5/12	0.81	7/13	5/3	5/9	6/6	0.66
BMI (kg/m^2)	25.8±4.7[*,†]	21.7±2.7[*]	22.1±4.5[†]	22.3±3.0	0.01	27.0±8.1	27.4±4.1	24.0±7.3	20.7±4.9	0.06
sBP (mmHg)	112.5±16.9	128.7±24.1	121.4±19.9	128.1±21.5	0.13	121.3±21.7	126.0±13.8	131.0±17.7	122.0±22.2	0.63
dBP (mmHg)	69.9±9.7	72.9±12.9	69.9±9.9	69.3±8.8	0.78	69.1±14.5	70.8±8.5	74.7±9.0	62.9±9.8	0.12
HR (bpm)	71.0±15.8	68.4±9.8	74.8±10.4	74.2±11.1	0.46	74.5±14.3	76.1±7.7	74.5±13.6	73.5±15.3	0.98
BT (°C)	36.0±0.7	36.0±0.8	36.3±0.5	36.0±0.6	0.54	35.9±0.5[‡]	35.8±0.4[§]	36.4±0.3[§,‡]	35.9±0.8	0.048
T-Chol (mg/dl)	213.8±73.9	219.7±29.9	204.9±55.1	204.6±51.7	0.83	219.5±58.4	237.6±49.6	177.7±35.4	179.7±37.4	0.16
LDL-C (mg/dl)	125.8±30.8	139.0±26.1	117.9±46.7	125.3±41.5	0.68	130.2±45.2	135.7±31.9	106.5±38.8	98.0±33.2	0.13
HDL-C (mg/dl)	61.6±24.7	66.8±15.2	68.8±19.5	62.3±19.7	0.84	56.1±17.9	63.7±8.5	53.9±18.4	57.5±30.6	0.88
TG (mg/dl)	138.9±71.3	126.1±81.8	124.6±78.0	156.1±127.8	0.78	157.1±104.2	150.3±73.4	137.8±53.4	147.6±94.8	0.78
L-T_4 dose (μg/day)	79.8±35.1[*]	108.9±30.4[‖]	51.3±46.2[*,‖]	–	<0.001	58.4±25.8[‡,‖]	106.3±11.6[‖,¶]	26.8±37.0[‡,¶]	–	<0.001
L-T_4 dose (μg/day per kg) (per body weight)	1.2±0.7[*]	2.1±0.7[*,‖]	0.9±0.6[‖]	–	<0.001	0.8±0.4[‡,§]	1.5±0.3[‡,‖]	0.4±0.6[§,‖]	–	<0.001

[a]*P* values are for the comparisons between all groups by ANOVA, except for sex distribution (χ^2 test), followed by Bonferroni's multiple comparison test between each of the two groups. *,†$P<0.05$, ‡,§$P<0.01$, ‖,¶$P<0.001$.

therapy, and the degree of TSH deficiency. Other pituitary hormone deficiency and their replacement therapy have been shown to affect the T_4 to T_3 conversion (14). In particular, GH replacement therapy promotes peripheral T_4 to T_3 conversion (10, 11). Indeed, in patients with CeH, serum FT_3 levels were higher in subjects with GH replacement therapy than those without it in our study. Coexisting GHD might decrease serum FT_3 levels because of decreased deiodinase acivity (20, 21). Furthermore, it is well known that high-dose glucocorticoid replacement therapy inhibits deiodinase activity (12, 13). However, there were few patients who had received such high-dose glucocorticoid treatment in our study and we did not find any differences in serum thyroid hormone levels between CeH patients with and those without ACTH deficiency. In addition, we should consider an influence of Gn on BT in patients with CeH. It has been reported that central estradiol upregulates Brown adipose tissue (BAT) thermogenesis via inactivating hypothalamic AMPK (22). However, we did not find any differences in BT or serum thyroid hormone levels between CeH patients with and those without menopause and hypogonadism.

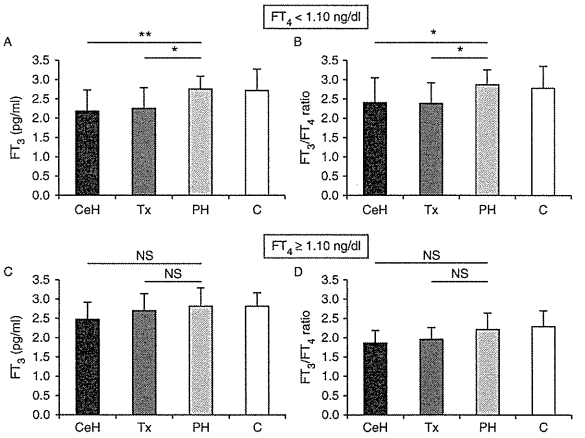

Figure 2

Serum FT_3 levels (A and C) and FT_3/FT_4 ratios (B and D) between four groups (subjects stratified according to the median-normal value of serum FT_4). Patients with CeH and Tx exhibited lower serum FT_3 levels (A) and FT_3/FT_4 ratios (B) than those with PH when serum FT_4 levels were below the median value of the normal range. Meanwhile, there were no significant differences in patients whose serum FT_4 levels were in the median-upper normal range (C and D). Data are expressed as mean±S.D. *P* values are for the comparisons between all groups by ANOVA, followed by a non-paired *t*-test between each of the two groups. *$P<0.05$, **$P<0.01$, NS, not significant.

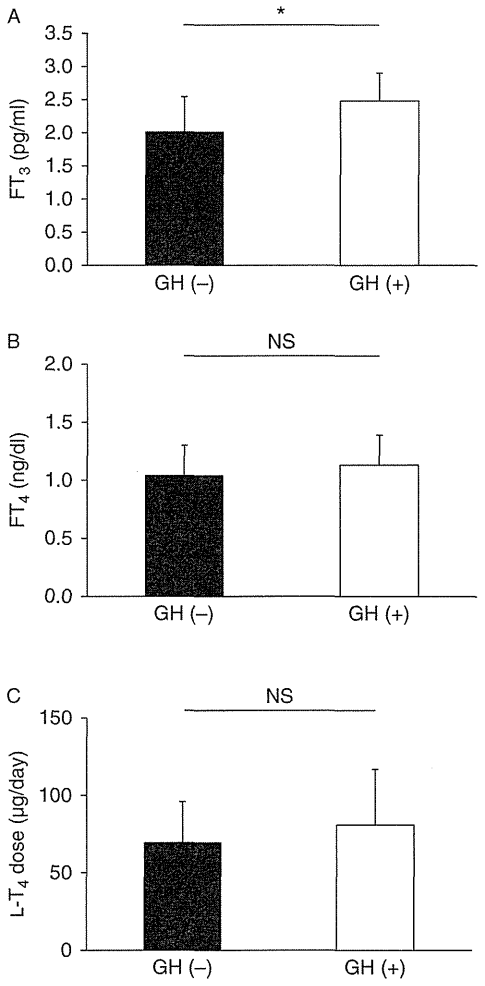

Figure 3

Serum FT_3 (A) and FT_4 (B) levels, and the dose of L-T_4 (C) in CeH patients with or without GH replacement therapy. In patients with CeH, serum FT_3 levels were significantly higher in patients with GH replacement therapy (GH (+)) than those without it (GH (−)), whereas serum FT_4 levels did not differ between these two groups. The dose of L-T_4 tended to be higher in the GH (+) patients than in the GH (−) patients. Data are expressed as mean±S.D. *P* values are for the comparisons between two groups by non-paired *t*-test. *$P<0.05$, NS, not significant.

It is also well known that hypothalamic damage can contribute to BT via the autonomic nervous system. However, in our study, patients who might suffer with hypothalamic damage by tumor invasion or treatment including surgery and/or radiotherapy were excluded. Furthermore, it has been reported that obesity is associated with BT or serum thyroid hormone levels (23, 24).

Table 5 BT and thyroid parameters according to BMI, menopausal status, and other pituitary hormone deficiency in CeH. Data are expressed as mean±S.D. *P* values are for the comparisons between two groups by using a non-paired *t*-test.

	BMI			Menopause			ACTH deficiency			Hypogonadism		
	≥25	<25	*P* value	(−)	(+)	*P* value	(−)	(+)	*P* value	(−)	(+)	*P* value
BT (°C)	36.1±0.8	36.0±0.6	0.62	36.2±0.5	35.9±0.8	0.20	36.0±0.2	36.0±0.8	0.91	35.9±0.5	36.1±0.8	0.43
FT_4 (mg/dl)	1.09±0.25	0.98±0.25	0.21	1.12±0.23	0.96±0.25	0.07	1.14±0.29	1.02±0.25	0.46	1.12±0.22	1.01±0.26	0.30
FT_3 (mg/dl)	2.25±0.53	1.94±0.47	0.09	2.30±0.51	1.91±0.47	0.053	2.35±0.26	2.04±0.54	0.11	2.09±0.45	2.08±0.54	0.98
FT_3/FT_4 ratio	2.19±0.80	2.04±0.56	0.55	2.16±0.77	2.07±0.61	0.72	2.16±0.53	2.10±0.70	0.85	1.89±0.38	2.17±0.73	0.19
L-T_4 dose (μg/day)	78.3±26.5	61.8±35.8	0.14	78.6±37.8	62.5±26.4	0.19	93.8±51.5	66.1±28.4	0.36	66.1±51.4	70.5±26.2	0.83
L-T_4 dose (μg/day per kg)	1.0±0.3	1.2±0.7	0.21	1.1±0.8	1.1±0.4	0.82	1.6±1.4	1.0±0.4	0.46	1.2±1.1	1.1±0.4	0.78

We analyzed the influence of obesity on BT or thyroid parameters in CeH but found no significant differences between CeH patients with and those without obesity.

Thyroid hormone is one of the key regulators of thermal homeostasis (25). T_3 induces uncoupling protein-1 (*UCP-1*) expression and mitochondrial biogenesis in human adipocytes, and the effects of T_3 on *UCP-1* induction are dependent on the thyroid hormone receptor-β (26). BAT is specialized for energy expenditure through thermogenesis (27), mediated by *UCP-1* expression (28). It is also known that the type 2 iodothyronine deiodinase (DIO2), an important enzyme to convert T_4 to T_3, is essential for adaptive thermogenesis in BAT (29). These findings along with our results indicate that BT might be reduced by relatively low serum FT_3 levels through low *UCP-1* expression levels in BAT.

The effect of combined therapy with L-T_4 and liothyronine (L-T_3) has been investigated for patients with hypothyroidism, whose QOL has been impaired with L-T_4 monotherapy. Several studies have compared the effect of L-T_4/L-T_3 combined therapy and that of L-T_4 monotherapy in patients with hypothyroidism. Bunevicius *et al.* (30) reported that partial substitution of L-T_3 for L-T_4 might improve mood and neuropsychological function in patients with both PH and Tx, suggesting the significance of additional L-T_3 administration. However, L-T_4/L-T_3 combined therapy demonstrated no beneficial changes in body weight, lipid profiles, and symptoms of hypothyroidism compared with L-T_4 monotherapy in patients with PH and Tx (31). Overall, L-T_4/L-T_3 combined therapy provided no advantage when compared with standard L-T_4 monotherapy in a meta-analysis of randomized controlled trials (32). However, because patients with CeH have not been included in these studies, L-T_4/L-T_3 combined therapy in patients with CeH is worth investigating.

To date, no consensus guidelines on the management of patients with CeH have been established. Several studies have been performed to investigate the optimal dose of L-T_4 in patients with CeH. It has been shown that L-T_4 dose based on body weight and aiming at serum FT_4 levels in the upper reference range is superior to aiming at the middle of normal FT_4 levels (2). In clinical practice, it has been recommended that serum FT_4 levels should be targeted within the middle to upper limit of the reference range in patients with CeH (7, 8, 9). However, there has been little evidence supporting the target levels in patients with CeH by using clinical markers of hypothyroidism, such as HR, BT, and lipid profiles during L-T_4 replacement therapy. Our results show that median-lower normal levels of serum FT_4 are associated with both low serum FT_3 levels and low BT, suggesting that these parameters could be clinically useful markers in patients with CeH, in addition to serum FT_4 levels. To confirm these findings, further investigations are required based on the other clinical markers such as basal metabolic rate measured by using an expiration gas analyzer or patient well-being assessed by using hypothyroid specific QOL questionnaires.

Our study has several limitations. First, the sample size, especially after stratification, was relatively small. Therefore, we could not exclude coincidental results. However, the association between low serum FT_3 levels and BT in patients with CeH strongly suggests a functional relevance. Second, various L-T_4 doses were used for replacement therapy, suggesting that patients with varied degrees of the remaining function of the pituitary's TSH secretion and the thyroid's T_3 or T_4 secretion were included, which might have affected the FT_3/FT_4 ratio. Third, although significant differences in serum FT_3 levels and FT_3/FT_4 ratios were observed in the patients with CeH and FT_4 <1.10 ng/dl, a similar tendency was found in the patients with CeH and FT_4 ≥ 1.10 ng/dl, suggesting that the threshold may not be the median-normal value of serum FT_4. In this aspect, the optimal replacement dose should be considered based on individual conditions. Fourth, several other confounding factors, including DIO2 polymorphism, may influence BT or serum thyroid hormone levels (33). Finally, the selection bias in each group needs to be considered. In particular, higher serum TSH levels were observed in patients with Tx because of the exclusion of subjects with TSH levels <0.4 μiu/ml, in whom TSH suppression therapy for thyroid cancer was performed.

In conclusion, patients with CeH who exhibited median-lower normal levels of serum FT_4 revealed low BT with relatively low serum FT_3 levels. GHD might have contributed to the serum FT_3 attenuation in these patients. These data support the previously recommended target levels of serum FT_4 at the middle to upper limit of the reference range in patients with CeH. It is not known whether the middle-upper normal levels of serum FT_4 would improve low BT and maintain well-being during L-T_4 replacement therapy in patients with CeH. Further large-scale, prospective, interventional studies are needed to determine the optimal replacement therapy in patients with CeH.

Declaration of interest
The authors declare that no conflict of interest could be perceived as prejudicing the impartiality of the research reported.

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Author contribution statement
Y Hirata drafted the manuscript, and assembled and analyzed the data. H Fukuoka and Y Takahashi were responsible for the conception and design of the study. The other coauthors contributed by collecting the data or caring for the patients. H Fukuoka was responsible for the critical revision of the manuscript for important intellectual content.

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The quality of life in acromegalic patients with biochemical remission by surgery alone is superior to that in those with pharmaceutical therapy without radiotherapy, using the newly developed Japanese version of the AcroQoL

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Abstract
Purpose To develop a Japanese version of the acromegaly quality of life (QoL) questionnaire (AcroQoL) and investigate the factors associated with impaired QoL in patients with acromegaly.
Methods We developed a Japanese version of the AcroQoL by a forward–backward method and evaluated QoL in 38 patients with acromegaly who had been followed up at an outpatient clinic at Kobe University Hospital. Its reliability was examined with Cronbach's alpha and item–total correlations. Second examination was performed for concurrent validity by assessment of correlations with the Short Form-36 (SF-36) and longitudinal analysis of the AcroQoL in 25 patients.
Results Cronbach's alpha and item–total correlations showed a range of 0.76–0.93 and 0.20–0.84, respectively, and significant correlations were found between the AcroQoL and the SF-36. Younger age and a history of radiotherapy were associated with worse total score by the multivariate linear regression analysis ($p = 0.020$ and $p = 0.042$, respectively). Intriguingly, in the biochemically-controlled group after the exclusion of patients who received radiotherapy, patients who underwent surgery alone exhibited a higher psychological (75.0 vs. 65.7 %, $p = 0.036$) and appearance (64.3 vs. 53.6 %, $p = 0.036$) score than those who are treating with pharmaceutical therapy.
Conclusions The reliability of the Japanese version of the AcroQoL was satisfactory. Younger age and a history of radiotherapy were associated with lower QoL in patients with acromegaly. In biochemically-controlled acromegaly, patients who underwent surgery alone exhibited better QoL than those under pharmaceutical therapy.

Keywords Acromegaly · Japanese patients · Surgery · Pharmaceutical therapy · Remission

Introduction

Acromegaly is a chronic disease caused by an excessive secretion of growth hormone (GH) from pituitary adenomas, and is associated with comorbidities such as hypertension, diabetes mellitus, osteoarthritis, sleep apnea syndrome, and changes in facial and acral appearances, and increased mortality. These comorbidities can impair quality of life (QoL) even when biochemical control is achieved with treatment [1–3].

There are 3 therapeutic approaches for acromegaly: surgery, pharmaceutical therapy, and radiotherapy [4]. With recent developments in therapies, more patients are biochemically-controlled than previously. A recent study showed that over 70 % of patients have normalized serum insulin-like growth factor I (IGF-I) levels after treatment [5]. Although normalization of GH and IGF-I levels improves the long-term mortality rate [6], self-perceived QoL of patients may not necessarily improve to the same

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degree [7–11]. The persistent impairment in QoL have been associated with several factors [12], among which a history of radiotherapy [2, 13–15].

The acromegaly QoL questionnaire (AcroQoL) is a disease-specific QoL questionnaire that was first developed in Spanish [16] and translated into many languages but not into Japanese until now. In this study, we have newly developed a Japanese version of the AcroQoL, validated its reliability, and investigated factors associated with QoL in Japanese patients with acromegaly.

Patients and methods

Study procedure

This study was approved by the Kobe University Ethics Committee, and written informed consent was obtained from all subjects. At first examination, patients were asked to answer the Japanese version of the AcroQoL questionnaire voluntarily at their clinical visit. At second examination, patients were asked to answer the AcroQoL questionnaire again as well as the Short Form-36 (SF-36) questionnaire 1 year after first examination. Both first and second examinations were set during 2 months.

Patients

Thirty-eight patients with acromegaly who had visited Kobe University Hospital for routine care were enrolled in first examination. They divided according to biochemical control status into three groups: controlled (n = 26), discordant (n = 7), and active (n = 5). Furthermore, the biochemically-controlled group was further divided according to treatment strategy into two groups after the exclusion of patients who received radiotherapy (n = 4): patients who had been controlled with surgery alone (n = 12) and those controlled with current pharmaceutical therapy (n = 10). Twenty-five (66 %) of 38 patients visited our outpatient clinic within the study period, and all of them completed second examination. Residual 13 patients did not visit because of following at another hospital, hospitalization due to other disease, or their own reasons. Patients who had severe liver dysfunction, renal dysfunction, or heart failure were excluded.

Endocrinological evaluation

The diagnosis of acromegaly was based on clinical signs, lack of serum GH suppression to <1 ng/mL during a 75 g oral glucose tolerance test, elevated serum IGF-I levels over the normal range for age- and sex-matched individuals, and the presence of pituitary tumors [17]. The duration of the disease was assessed visually through comparison of photographs and the onset of related symptoms as previously described [18, 19]. Patients underwent transsphenoidal surgery that yielded a histological diagnosis of GH-producing pituitary adenoma. Clinical data were retrospectively collected from patients' medical records. Random serum GH and IGF-I were measured in the morning after overnight fasting. Serum GH levels were measured with an enzyme-linked immunosorbent assay (ELISA, Tosoh Co. Ltd., Tokyo, Japan), and serum IGF-I level with an immunoradiometric assay (IRMA, Daiichi Radioisotope Laboratories, Tokyo, Japan). The IGF-I standard deviation (SD) score was calculated based on age- and sex-matched healthy Japanese individuals [20]. Random GH <1 ng/mL and/or IGF-I SD score <2 were used for biochemical control criteria as previously described [21]. Based on the data during the visit period, patients were divided into three groups: patients who met both criteria of GH and IGF-I were defined as "controlled", those who met either of the two criteria were defined as "discordant", and those who met neither criteria were defined as "active". Two patients who were treated with pegvisomant were assessed only with the IGF-I SD score.

Development of a Japanese version of the AcroQoL

The AcroQoL consists of 22 items and is divided into two main categories: physical (eight items) and psychological (14 items) scale. The psychological scale is further divided into two subscales: appearance (seven items) and personal relationship (seven items). The frequency of occurrence and degree of agreement with the items were selected as response choice using a five-point Likert scale. The total score ranges from 0 to 100, with a higher score indicating better QoL [16].

The original Spanish version was first translated into Japanese by two independent professional bilingual translators, who had experience in translating health-related QoL questionnaires. The first versions by these translators were compared with each other and with the original Spanish version at a consensus meeting to produce the first intermediary Japanese version of the questionnaire. This version was independently translated back into Spanish to ascertain equivalent significance in both languages. After a second meeting, the second intermediary Japanese version was produced and presented to five Japanese patients with acromegaly to assess and correct for comprehension, clarity, cultural relevance, and suitable wording (cognitive debriefing); then, the final Japanese version of the AcroQoL questionnaire was provided.

SF-36 questionnaire

The SF-36 questionnaire has been widely used in many countries, and the Japanese version has been approved [22]. The Japanese version of the SF-36 comprises 36 items and evaluates general well-being during the previous 30 days. The items are formulated as statements or questions to assess eight health concepts: (1) physical functioning, (2) role physical, (3) bodily pain, (4) general heath, (5) vitality, (6) social functioning, (7) role emotion, and (8) mental health. Furthermore, it consists of three component summary scores: (1) physical component summary (PCS), (2) mental component summary (MCS), and (3) role/social component summary (RCS) [23]. Scores range from 0 to 100, with a higher score indicating a better QoL.

Study design

The Japanese version of the AcroQoL score was evaluated in all patients, and correlations with their clinical characteristics were analyzed. The scores were compared between the three different biochemical control groups: controlled, discordant, and active. In addition, the scores between biochemically-controlled groups by different treatment strategies were also compared. Since lower AcroQoL scores have been shown in patients treated with radiotherapy [2, 13–15], patients with a history of radiotherapy were excluded from this analysis.

Statistics

All statistical analyses were performed using the SPSS Statistics version 22 software package (IBM Inc., Chicago, IL, USA). Reliability and internal consistency of the Japanese version of the AcroQoL were evaluated with Cronbach's alpha and item–total correlation. Cronbach's alpha $\geqq 0.7$ and item–total correlation $\geqq 0.2$ were considered satisfactory [24, 25]. Concurrent validity was assessed with correlations between the AcroQoL and the SF-36 in a second examination group of 25 patients. The correlation between nonparametric data was assessed with Spearman's rank correlation in Figs. 1 and 2. Kruskal–Wallis test was used in Table 3. For continuous variables, differences were analyzed using the Mann–Whitney U test for nonparametric data, and for categorical variables, differences were analyzed using the χ^2 test and Fisher's exact test in Table 4. In multivariate linear regression analysis, age, sex, duration of disease, IGF-I SD score, and history of radiotherapy were included in order to identify the independent correlates of AcroQoL total score and each subscale score. Statistical significance was accepted at $p < 0.05$. AcroQoL scores are expressed as median and range, and other data are shown as mean ± SD.

Results

Patient characteristics

Clinical characteristics of all 38 patients are shown in Table 1. The mean age at evaluation was 56.1 ± 12.6 years, and the male to female ratio was 1:1. The mean disease duration was 17.4 ± 9.0 years. Mean serum GH levels, IGF-I levels, and IGF-I SD score were 2.08 ± 3.59, 174.6 ± 65.3, and 0.80 ± 1.50 ng/mL, respectively. Twenty-six patients (68.4 %) had biochemically-controlled disease, seven patients (18.4 %) had discordant results, and five patients (13.2 %) had active disease.

Thirty-two patients (84.2 %) had undergone transsphenoidal surgery, whereas four patients (10.5 %) had received radiotherapy. Twenty-five patients were on pharmaceutical therapy at the time of evaluation (16 patients; somatostatin analogs alone; five patients, both somatostatin analogs and dopamine agonists; two patients, dopamine agonists alone; one patient, both somatostatin analogs and pegvisomant; and one patient, pegvisomant alone).

Validation of Japanese AcroQoL

Cronbach's alpha and item–total correlation, which evaluated by all 38 patients' data, in the total score and each subscale score showed a range of 0.76–0.93 and 0.20–0.84, respectively (Table 2), indicating satisfactory indices. In the analysis of the second examination group of 25 patients data, the validity of the Japanese version of the AcroQoL was reinforced by correlations with the SF-36: between the physical scale in the AcroQoL and the PCS score in the SF-36 ($r = 0.491$, $p = 0.013$), and the psychological scale in the AcroQoL and the MCS score in the SF-36 ($r = 0.751$, $p < 0.001$) (Fig. 1).

AcroQoL outcome

In all 38 patients with acromegaly, median total scores, physical scale, and psychological scale on AcroQoL were 67.0 % (range 31.8–96.6 %), 67.2 % (range 18.8–100.0 %), and 67.9 % (range 28.6–94.6 %), respectively. Subscale appearance and subscale personal relationship were 57.1 % (range 21.4–92.9 %) and 78.6 % (range 32.1–100.0 %) respectively (Table 2). Multivariate linear regression analysis identified younger age ($p = 0.020$) and a history of radiotherapy ($p = 0.042$) to be associated with worse total score. In subanalysis, younger age ($p = 0.026$) and a history of radiotherapy ($p = 0.045$) were associated with worse physical and only younger age was associated with worse physiological scale ($p = 0.049$). In the longitudinal analysis, the changes in the

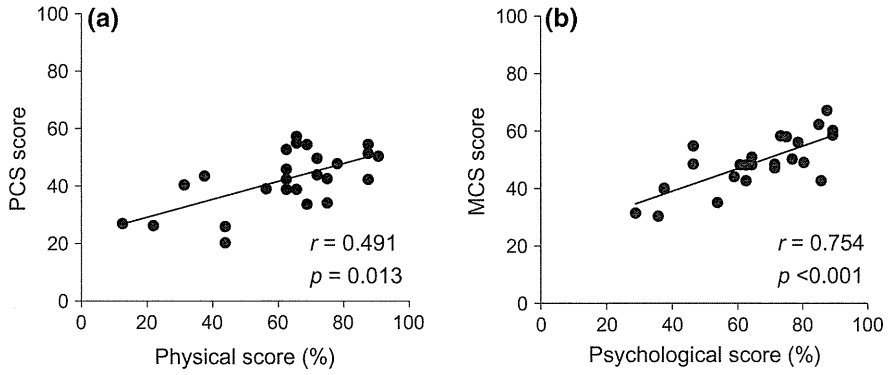

Fig. 1 Correlation analysis between AcroQoL scores and SF-36 scores. **a** Physical scores and PCS scores, **b** psychological scores and MCS scores

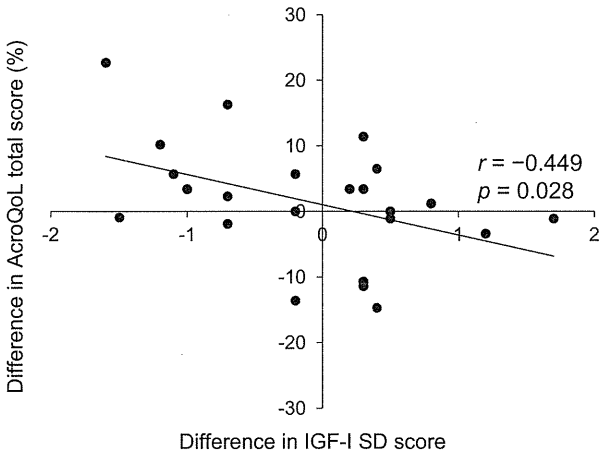

Fig. 2 Correlation analysis between change in IGF-I SD scores and change in AcroQoL total scores

IGF-I SD score were negatively correlated with those in the AcroQoL total score (Fig. 2, $r = -0.449$, $p = 0.028$).

AcroQoL scores according to disease activity of acromegaly

We divided all patients into three groups according to disease activity: 26 controlled, seven discordant, and five active according to serum GH and IGF-I levels, and compared the QoL score between these groups (Table 3). There was no significant difference between groups, although the physical score in the active group tended to be lower.

Effect of surgery or pharmaceutical therapy on AcroQoL scores in disease-controlled patients

Age, sex, duration of the disease, GH, IGF-I SD score, and the presence of hypopituitarism did not differ between groups. Intriguingly, patients who had undergone surgery alone exhibited a higher psychological score (75.0 vs. 65.7 %, $p = 0.036$), especially in the appearance score

Table 1 Clinical characteristics of the patients with acromegaly enrolled in this study

Variables	
Total number of patients (N)	38
Age (years)	56.1 ± 12.6
Male/female (N)	19/19
Disease duration (years)	17.4 ± 9.0
GH (ng/mL)	2.08 ± 3.59
IGF-I (ng/mL)	174.6 ± 65.3
IGF-I SD score	0.80 ± 1.50
Previous treatment (N)	
Surgery alone	29
Radiotherapy alone	1
Surgery + radiotherapy	3
None	5
Current treatment (N)	
Somatostatin analog	16
Dopamine agonist	2
Pegvisomant	1
Combination of two agents	6
None	13
Treatment of hypopituitarism (N)	
Hydrocortisone alone	2
Sex steroid alone	2
Hydrocortisone + levothyroxine	1

Data are shown by mean ± SD

Table 2 Cronbach's alpha and item–total correlation in the Japanese version of the AcroQoL

	Cronbach's alpha	Item–total correlation
Total	0.93	0.20–0.84
Physical	0.88	0.37–0.80
Psychological	0.88	0.20–0.77
Appearance	0.76	0.23–0.77
Personal relationships	0.80	0.40–0.72

Table 3 AcroQoL scores in all patients and according to acromegaly disease activity (median and range)

	All (n = 38)	Controlled (n = 26)	Discordant (n = 7)	Active (n = 5)	*p* value (between three control groups)
AcroQoL score (%)					
Total	67.0 (31.8–96.6)	67.0 (31.8–96.6)	68.2 (35.2–92.0)	55.7 (42.0–78.4)	0.837
Physical	67.2 (18.8–100.0)	68.8 (31.3–100.0)	71.9 (21.9–96.9)	43.6 (18.8–84.4)	0.461
Psychological	67.9 (28.6–94.6)	68.8 (28.6–94.6)	69.6 (37.5–89.3)	67.9 (55.4–78.6)	0.746
Appearance	57.1 (21.4–92.9)	57.1 (21.4–89.3)	57.1 (32.1–92.9)	60.7 (35.7–71.4)	0.536
Personal relationships	78.6 (32.1–100.0)	78.6 (32.1–100.0)	82.1 (42.9–92.9)	78.6 (64.3–96.4)	0.791

Table 4 Clinical characteristics and AcroQoL scores in biochemically-controlled patients with acromegaly

	(a) Surgery only (n = 12)	(b) On medical therapy including radiotherapy (n = 14)	(c) On medical therapy without radiotherapy (n = 10)	*p* value (a) versus (b)	*p* value (a) versus (c)
Age (years)	58.3 ± 10.3	54.7 ± 14.4	53.9 ± 15.6	0.374	0.381
Male/female (N)	7/5	5/9	4/6	0.431	0.670
Duration of disease (years)	14.4 ± 5.8	20.6 ± 8.7	20.8 ± 9.6	0.403	0.497
Duration under control (years)	4.7 ± 3.7	4.9 ± 3.6	5.5 ± 3.5	1.000	0.605
GH (ng/mL)	0.88 ± 0.78	0.87 ± 0.32	0.86 ± 0.31	0.514	0.602
IGF-I SD score	0.41 ± 1.32	−0.13 ± 1.4	0.30 ± 1.08	0.860	0.705
Radiotherapy	0 (0 %)	4 (29 %)	0 (0 %)	0.100	–
Hypopituitarism (N)	2 (17 %)	0 (0 %)	0 (0 %)	0.203	0.481
DM (N)	1 (8.3 %)	5 (36 %)	3 (30 %)	0.170	0.293
HTN (N)	5 (42 %)	5 (36 %)	3 (30 %)	1.000	0.675
DL (N)	7 (58 %)	2 (14 %)	2 (20 %)	0.038*	0.099
SAS (N)	3 (25 %)	2 (14 %)	1 (10 %)	0.586	0.594
OA (N)	2 (17 %)	1 (7 %)	0 (0 %)	0.635	0.481
AcroQoL score (%)					
Total	75.6 (56.8–96.6)	61.9 (31.8–84.1)	64.6 (31.8–84.1)	0.009*	0.069
Physical	75.0 (34.4–100.0)	59.4 (31.3–87.5)	67.2 (34.4–87.5)	0.046*	0.228
Psychological	75.0 (58.9–94.6)	60.3 (28.6–85.7)	65.7 (28.6–85.7)	0.005*	0.036*
Appearance	64.3 (35.7–89.3)	53.6 (21.4–78.6)	53.6 (21.4–78.6)	0.015*	0.036*
Personal relationships	87.5 (67.9–100.0)	67.9 (32.1–92.9)	75.0 (32.1–92.9)	0.031*	0.159

DM diabetes mellitus, *HTN* hypertension, *DL* dyslipidemia, *SAS* sleep apnea syndrome, *OA* osteoarthritis

* $p < 0.05$

(64.3 vs. 53.6 %, $p = 0.036$), than patients who received pharmaceutical therapy (Table 4).

Discussion

In this study, we developed a Japanese version of the AcroQoL for the first time and tested whether this questionnaire is useful to evaluate the QoL in Japanese patients with acromegaly. Cronbach's alpha and item–total correlation, the scales of reliability and internal consistency, respectively, for the Japanese version were comparable with those of the original Spanish version [16]. The distribution of AcroQoL scores in this study was compatible with that of previous studies particularly in the subscale of appearance [3, 8, 9, 16, 26, 27]. In addition, the close correlations between the Japanese version of the AcroQoL scores and SF-36 component summary scores reinforce the validity of the Japanese version of the AcroQoL. Furthermore, we showed a negative correlation between the change in IGF-I levels and AcroQoL scores as described previously [9]. These results indicate that this Japanese

version of the questionnaire effectively evaluated the QoL of Japanese patients with acromegaly.

We clearly demonstrated that QoL is different in biochemically-controlled patients with acromegaly according to the therapeutic method; patients treated with surgery alone exhibited higher QoL than those under pharmaceutical therapy. Generally, achieving a biochemically-controlled status only by surgery is superior to the continuous pharmaceutical therapy required for this status. Furthermore, the economic burden and inconvenience of pharmaceutical therapy could be of concern to patients with acromegaly [28]. We speculate that these disadvantages could deteriorate the comprehensive QoL in patients receiving pharmaceutical therapy, even if their physical condition is not impaired in a biochemically-controlled status. However, these should affect QoL not only in appearance but also in personal relationships. Our results demonstrated that the appearance score in particular was lower in patients with pharmaceutical therapy than in those with surgery alone, suggesting that there might be more complex factors involved. One possibility to explain these results is that the burden of disease could indirectly impair QoL. Indeed, illness perception and treatment satisfaction are associated with QoL in patients with acromegaly [28, 29]. Such factors could affect the psychological condition indirectly but perhaps affect not only the appearance score. In fact, biochemically-controlled acromegalic patients are more depressive than the general population [30], and the necessity of pharmaceutical therapy is associated with a depressive state [31, 32]. It has also been reported that some patients need to take "drug holidays" just to feel free from the disease and its treatment [33]. We speculate that a depressive mood associated with life-long pharmaceutical therapy could become a liability for their body images. Another plausible and interesting possibility is that the improvement effect on appearance differs between the 2 therapeutic methods. The surgery normalizes serum GH and IGF-I levels more rapidly [34] than pharmaceutical therapy [35, 36]. This may result in relatively rapid changes in appearance, and the patients perceive the improvement more clearly than those who undergo pharmaceutical therapy.

In line with our results, Hua et al. [10] reported that biochemically-controlled acromegaly patients treated with lanreotide showed worse QoL scores in total and psychological scales of the AcroQoL than those treated without lanreotide. Additionally, Postma et al. [37] reported that, despite similar IGF-I SD scores, QoL in patients with acromegaly was impaired in association with the need for prolonged postoperative therapy with somatostatin analogs. However, these reports included substantial proportions of patients who had been treated with radiotherapy and those who received hormone replacement therapy; thus, it is difficult to exclude the possibility that these factors affected QoL. On the other hand, another previous report demonstrated no significant difference in the QoL score between biochemically-controlled patients who had undergone surgery and those treated pharmaceutically without surgery [27]. Although the precise reason for this discrepancy is unknown, the influence of radiotherapy on QoL needs to be considered. In fact, the patients who had undergone surgery had more history of radiotherapy than those treated pharmaceutically without surgery in this previous study. In contrast, in the current study, we excluded patients treated with radiotherapy because several reports demonstrated that radiotherapy itself is associated with impaired QoL [2, 13–15]. The exclusion of these subjects in our study clearly reveals the differences between the effects of surgery and pharmaceutical therapy on QoL. Current consensus has recommended that pharmaceutical therapy is an option for primary treatment if surgery is not appropriate, indicating that surgery is the first option when cure is expected [38]. Our data supports this recommendation from a QoL point of view. Recently, it has been reported that using pharmaceutical therapy as a first-line treatment instead of surgery is an option for achieving biochemical control [39]; however, the present data suggest the importance of QoL when a treatment option is selected.

We demonstrated that younger acromegalic patients showed a lower total score on the AcroQoL. The decreased QoL of these subjects was associated with both physical and psychological scales. Furthermore, the appearance scale also tended to decrease with younger age (data not shown). Generally, perception of appearance affects relationships and social activities mostly during the late teens and 20s. For example, according to a population-based study, the prevalence of body dysmorphic disorder is most frequent in the late teens and 20s [40]. Another study reported that the prevalence of concern with appearance is higher in younger age groups [41]. These results might suggest that these specific characteristics of younger patients affect QoL. A lower QoL in younger patients compared with that in elderly patients was reported in breast and lung cancer survivors [42, 43]. In addition, diagnosis of type 2 diabetes at a younger age was associated with a poor QoL [44]. In these paper, various possible reasons for worse QoL in younger age was described, including greater responsibilities or expectations for their health when they are diagnosed, and greater concern for their diseases. These reasons could be applicable in our patients with acromegaly. In contrast to our results, a couple of previous studies showed no age-related correlation in AcroQoL score [8, 13, 45]. One possible explanation for this discrepancy is that the proportion of patients who received radiotherapy and who had hypopituitarism

was quite low in our study. Typically, adverse events of radiotherapy, including hypopituitarism, occur more than 5–10 years after treatment [46, 47]. Patients who received radiotherapy have been reported to have impaired QoL during follow-up despite biochemically-controlled status of acromegaly [14]. In previous QoL studies for acromegaly, 38.6–67.6 % of patients received radiotherapy [8, 13, 45], which could offset the QoL of older patients.

There are several limitations in this study. First, the analysis was mainly performed in the cross-sectional design without a control group and not all the patients were evaluated by the longitudinal data collection. Second, a relatively small sample size decreased the statistical power. This is also the reason why construct validity could not be assessed with a factor analysis. Furthermore, the number of patients with biochemically discordant or active disease is limited and IGF-I SD scores of patients with active disease were not so high (median 3.1, range 2.1–3.7), which might decrease the power to detect differences in QoL between the groups. Although significant differences in QoL were detected between patients with or without biochemical control in a couple studies [8, 27], other studies reported that there were no differences depending on the control status [10, 26]. These results may be explained by the multiple factors that could affect QoL [12] and the limitation of the cross sectional design of the study.

The strength of our study is that patients who had received radiotherapy were excluded in the analysis of biochemically-controlled patients. It has been reported that patients with pharmaceutical therapy showed decreased QoL scores; however, these studies included a substantial number of patients treated with radiotherapy, which could affect QoL [10, 27, 37]. In this study, we excluded patients with a history of radiotherapy and showed a decreased QoL in patients with pharmaceutical therapy compared with those with surgery alone, which was in line with the previous report [48].

In conclusion, we developed a Japanese version of the AcroQoL for the first time and showed its reliability. Younger age was associated with lower QoL in patients with acromegaly. In biochemically-controlled subjects, patients treated with surgery alone exhibit better QoL than those under pharmaceutical therapy. These results strengthen the importance of biochemical remission by surgery compared with that by current pharmaceutical therapy.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

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RESEARCH ARTICLE

Accelerated Telomere Shortening in Acromegaly; IGF-I Induces Telomere Shortening and Cellular Senescence

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Abstract

Objective

Patients with acromegaly exhibit reduced life expectancy and increased prevalence of age-related diseases, such as diabetes, hypertension, and cardiovascular disease. However, the underlying mechanism has not been fully elucidated. Telomere shortening is reportedly associated with reduced life expectancy and increased prevalence of these age-related diseases.

Methods

We measured telomere length in patients with acromegaly using quantitative PCR method. The effect of GH and IGF-I on telomere length and cellular senescence was examined in human skin fibroblasts.

Results

Patients with acromegaly exhibited shorter telomere length than age-, sex-, smoking-, and diabetes-matched control patients with non-functioning pituitary adenoma (0.62 ± 0.23 vs. 0.75 ± 0.35, respectively, $P = 0.047$). In addition, telomere length in acromegaly was negatively correlated with the disease duration ($R^2 = 0.210$, $P = 0.003$). *In vitro* analysis revealed that not GH but IGF-I induced telomere shortening in human skin fibroblasts. Furthermore, IGF-I-treated cells showed increased senescence-associated β-galactosidase activity and expression of p53 and p21 protein. IGF-I-treated cells reached the Hayflick limit earlier than GH- or vehicle-treated cells, indicating that IGF-I induces cellular senescence.

supported the clinical and in vitro analysis in the study.

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Conclusion

Shortened telomeres in acromegaly and cellular senescence induced by IGF-I can explain, in part, the underlying mechanisms by which acromegaly exhibits an increased morbidity and mortality in association with the excess secretion of IGF-I.

Introduction

Telomeres consist of repetitive DNA sequences, thousands of "TTAGGG" tandem repeats, which are located at the ends of linear chromosomes in most somatic cells [1]. Telomere ends form a cap-like structure to protect the ends of chromosomes from degeneration and fusion [2]. However, telomeres shorten during each cell division and when they reach a critically short length, cell cycle arrest and senescence occur; this is known as the "Hayflick limit" in cultured human cells [3]. Telomere damage activates DNA damage response (DDR), a signaling pathway in which cell cycle progression is blocked via an increased production of p53 and cyclin-dependent kinase (Cdk) inhibitor p21 protein [4]. DDR subsequently induces cellular senescence.

A number of observations suggest a close connection between telomere length and mortality and age-related disease [5]. Telomere length measured in peripheral leukocytes is related to mortality; subjects with shorter telomeres are more likely to succumb to cardiovascular disease and infectious diseases [6]. Furthermore, exposure to various stresses and age-related diseases such as diabetes, cardiovascular disease, and neurodegenerative disease are associated with shortened telomeres [7–9]. Smoking, obesity, hypertension, and atherosclerosis are also associated with shortened telomeres [10–12]. As an underlying mechanism, it has been reported that the increased oxidative stress enhances telomere DNA damage. Telomeres are rich in guanine residues and may be particularly sensitive to reactive oxygen species (ROS) because guanine can be oxidized to 8-hydroxyguanine, which is unstable [5].

Recent studies have focused on the relationship between telomere length and endocrine disorders. Patients with polycystic ovary syndrome reportedly exhibit a shortened telomere length [13]. Aulinas et al. reported that patients with active Cushing's syndrome showed shortened telomeres [14]. Although the potential relationship between telomere length and the growth hormone (GH) and insulin-like growth factor-I (IGF-I) axis has been discussed [15], to the best of our knowledge, telomere length in acromegalic patients has not been reported. Acromegaly is characterized by the over-secretion of GH, mostly caused by GH-producing pituitary adenomas. It is well known that patients with acromegaly have increased mortality, which is associated with comorbidities of age-related disease such as cardiovascular, cerebrovascular, respiratory, and malignant diseases [16, 17]. Although the increased morbidity and mortality is strongly associated with the degree of GH and IGF-I excess, the precise underlying mechanisms have not been fully elucidated.

Here, we examined the telomere length in peripheral leukocytes in patients with acromegaly and control patients with non-functioning pituitary adenoma (NFPA). In addition, we investigated the effect of GH and IGF-I on telomere length and cellular senescence to clarify the underlying mechanisms.

Materials and Methods

Patients

This study was approved by the Kobe University Hospital and Toranomon Hospital Ethics Committee and written informed consent was obtained from all subjects. We recruited 61

consecutive patients with acromegaly and 27 consecutive patients with NFPA who underwent transsphenoidal surgery at Toranomon Hospital between 2005 and 2010. The diagnosis of acromegaly and NFPA was based on their clinical findings, laboratory data, and imaging studies, and confirmed by pathological findings in the surgically removed tumors. The clinical data and blood samples for telomere length analysis were obtained before the surgery, thus all patients with acromegaly had an active disease. Seven patients with acromegaly received preoperative medical therapies (5 patients received somatostatin analogue, 1 patient received dopamine agonist, and 1 patient received both of them) and no one received radiotherapy. We excluded patients who had undergone previous pituitary surgery, patients with malignancy and other endocrine disorders, and patients in whom hormone replacement therapy (hydrocortisone, thyroxin, GH, gonadotropins, and/or gonadal steroid hormones) was necessary before surgery. We also excluded patients with NFPA whose IGF-I standard deviation score (SDS) was lower than −2.0.

Endocrinological evaluation

Endocrinological data were obtained before the surgery. The diagnosis of acromegaly was based on clinical signs, lack of serum GH suppression to < 1 ng/mL during a 75 g oral glucose tolerance test (OGTT), elevated serum IGF-I levels corresponding to the normal range for age- and sex-matched individuals, and the presence of pituitary tumors [18]. The duration of the disease was assessed visually by comparison of photographs and by the onset of related symptoms as previously described [18]. All patients underwent transsphenoidal surgery that yielded a histological diagnosis of GH-producing pituitary adenoma or NFPA. Clinical data were retrospectively collected from patients' medical records. Basal serum levels of GH and IGF-I were measured in the morning after overnight fasting. Serum GH and IGF-I levels were measured by an immunoenzymometric assay using the ST AIA-PACK hGH kit (TOSOH Corporation, Tokyo, Japan) and an immunoradiometric assay using "Daiichi" IGF-I IRMA kit (FUJIFILM RI Pharma Co.,Tokyo, Japan), respectively. Intra- and inter-assay coefficients of variation (C. V.) for the assay of GH and IGF-I were as follows: GH (intra-C.V. 1.3% and inter-C.V. 3.3%) and IGF-I (intra-C.V. 1.1% and inter-C.V. 2.2%), respectively.

Histological analysis

Surgically removed adenoma tissues were fixed in formaldehyde, embedded in paraffin, and cut into 3 μm thick sections for immunohistological staining. For GH immunostaining, anti-GH polyclonal antibody was used (Dako, Carpinteria, CA, USA; A0570). The diagnosis of acromegaly or NFPA was confirmed by the histological findings. NFPA was defined as an adenoma in which GH, PRL, ACTH, and TSH were negative.

Telomere length measurement

Leukocyte telomere length was examined in blood samples, which were collected before the surgery. Genomic DNA was extracted from peripheral leukocytes using the Gentra Puregene Blood Kit (QIAGEN, Venlo, Netherlands). The telomere length for each patient was determined using a quantitative PCR assay as previously described [19] with a slight modification. All assays were performed by the Step One Plus™ Real Time PCR system (Applied Biosystems, Tokyo, Japan) in a 96-well plate. Twenty ng of DNA was subjected to the PCR reaction. Each reaction well included 1× SYBR Premix Ex Taq™ II (TaKaRa Bio Inc., Shiga, Japan), 1.5 mM $MgCl_2$, 1 mM dithiothreitol, and 1 M betaine. The telomere quantity was normalized to β-globin gene. Each primer sequence and concentration in the reaction are shown in S1 Table. The thermal cycling profile was 15 min at 95°C; two repeats of 15 s at 94°C followed by 15 s at

49°C; 36 repeats of 15 s at 95°C, 120 s at 58°C, and 30 s at 74°C; followed by a melting curve analysis for verification of the PCR product. All samples were assayed in triplicate using a standard curve with 5 concentrations spanning an 81-fold range (100, 33.3, 11.1, 3.7, 1.23 ng) of standard DNA (obtained from a healthy 30-year-old man). The relative telomere length (RTL) was calculated as the ratio of the telomere repeat copy number to the single gene copy number (T/S) according to the standard curve. Two patients with RTLs over 2.0 were excluded from subsequent statistical analysis because they were outliers (a 57-year-old female in the Acro group with an RTL of 2.1, and a 41-year-old female in the NFPA group with an RTL of 2.1).

Cell culture and GH/IGF-I treatment

Human skin fibroblasts were obtained from a 17-year-old healthy man after obtaining written informed consent. Cells were cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum (FBS) and incubated at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Cells were treated with 100 or 500 ng/mL of recombinant human GH (Eli Lilly, Kobe, Japan), recombinant human IGF-I (Astellas Pharma Inc. Tokyo, Japan), or vehicle. The medium with these compounds was changed every other day. Cultured cells were passaged before reaching confluence and seeded at 3.5×10^3 cells/cm^2. Population doubling levels (PDL) were calculated as PDL = $\log_2(N_n/N_0)$, where N_n is the cell number at the passage and N_0 is the initial number of fibroblasts.

Quantitative reverse transcription PCR

Human fibroblasts at day 10 and 27 (PDL of 10–11 and 21–22) were used for these experiments (S1A Fig). The mRNA expression levels of p53 and p21 were quantified using qRT-PCR at day 10 and 27. That of Interleukine-6 (IL-6) was quantified at day27. Total RNA was extracted from the cells using TRI reagent (Molecular Research Center, Inc., OH, USA). Five-hundred ng of total RNA was subjected to reverse transcription using the ReverTra Ace qPCR RT Kit (TOYOBO, Osaka, Japan). All quantitative PCR reactions were performed with the Step One Plus™ Real Time PCR system (Applied Biosystems, Tokyo, Japan) using SYBR mix Ex Taq™ II (TaKaRa Bio Inc., Shiga, Japan). The thermal cycling profiles were as follows: initial denaturation at 95°C for 15 min, followed by 30 cycles of denaturation at 94°C for 15 sec, annealing at 55°C for 15 sec, and extension at 72°C for 15 sec. β-actin was used as an internal control. Each primer sequence used in the experiments is shown in S2 Table. All samples were assayed in duplicate. The representative results from four independent experiments are shown.

Immunoblotting

Human fibroblasts at day 10 and 27 (PDL of 10–11 and 21–22) were used for these experiments (S1A Fig). Cells were washed twice with PBS and lysed in lysis buffer containing 50 mM Tris HCl pH 7.5, 30 mM KCl, 5 mM EDTA, 1% NP-40, 1 mM dithiothreitol, and 0.1% sodium dodecyl sulfate (SDS), protease inhibitor (Nacalai Tesque, Kyoto, Japan), and phosphatase inhibitor (Pierce Thermo Fisher Scientific, IL, USA). Twenty μg of protein per lane was subjected to SDS-polyacrylamide gel electrophoresis (PAGE), and then transferred to a polyvinylidene fluoride membrane. The membrane was incubated with primary antibody overnight at 4°C. Antibodies for total p53 and phosphorylated p53 (Ser 15) were obtained from Cell Signaling Technology (Danvers, MA, USA). Anti-p21 antibody and anti-β-actin antibody were obtained from Santa Cruz Biotechnology (Dallas, TX, USA) and Sigma-Aldrich (St Louis, MO, USA), respectively. After incubation with horseradish peroxidase-conjugated secondary antibody for 1 hr at room temperature, signals were visualized with ImmunoStar LD (WAKO, Tokyo, Japan) or Chemi-Lumi One L solution (Nacalai Tesque, Kyoto, Japan).

Senescence-associated β-galactosidase staining

Senescence-associated β-galactosidase staining (SA β-Gal) was performed as previously described with a slight modification [20]. Human fibroblasts at day 38 (PDL of 28–29) were used for these experiments (S1A Fig). Briefly, cells were washed with PBS and fixed with 0.5% glutaraldehyde solution for 15 min. The cells were washed again two times with pH 5.5 PBS with 40 mM $MgCl_2$. Subsequently, staining solution (1 mg/mL 5-bromo-4-chloro-3-indolyl β-D-galactosidase (X-gal) in dimethylformamide, 5 mM potassium ferrocyanide, and 5 mM potassium ferricyanide dissolved in pH 5.5 PBS with 40 mM $MgCl_2$) was added and cells were incubated at 37°C for 8 hrs. After incubation, the cells were washed with PBS and photographed. The proportion of senescent cells stained blue was determined in 6 fields at 200× magnification.

Statistical analysis

Data are appropriately expressed as means ± standard deviations or medians [interquartile range]. Continuous data were compared by a Student's *t*-test or a Mann—Whitney test and categorical data were compared by a χ^2 test or Fischer's exact test, as appropriate. Multiple groups were compared by a one-way analysis of variance with a post-hoc Fischer's least significant difference test, a Kruskal—Wallis test with a post-hoc Scheffe test, or a χ^2 test followed by Tukey's honestly significant difference test, as appropriate. Homogeneity of variance was tested by Levene's test. Pearson's test was used to evaluate the correlation between two variables. *P*-values of 0.05 or less were considered significant. Statistical analyses were performed using JMP Statistical Database Software version 8. 0. 1 (SAS Institute, Inc. Cary, NC, USA).

Results

Telomere length in acromegaly

To investigate whether patients with acromegaly exhibit shortened telomeres, we compared them with age-, sex-, smoking-, and diabetes-matched patients with NFPA. Forty-six patients with acromegaly after the exclusion of the patients who met the exclusion criteria (the Acro group) and 20 out of 27 patients with NFPA (the NFPA group) were enrolled in this study. The clinical characteristics of these groups are shown in Table 1. As expected for the initial matching, there were no significant differences in age, sex ratio, and the ratio of smoking and diabetes between these two groups. Also, there were no statistical differences in BMI, HbA1c levels and the prevalence of hypertension and dyslipidemia. All patients in the NFPA group had a macroadenoma and the tumor diameter was significantly greater in the NFPA group than in the Acro group. As predicted, the preoperative serum random GH levels and IGF-I SDS were significantly higher in the Acro group than in the NFPA group. With respect to other pituitary hormones, although no significant differences were observed, TSH levels tended to be lower in the Acro group. RTL was evaluated in these groups. As shown in Fig 1A, RTL in the Acro group was significantly shorter than that in the NFPA group (0.62 ± 0.23 vs. 0.75 ± 0.35, $P = 0.047$).

To investigate the factors associated with telomere length in acromegaly, we evaluated the relationship between RTL and various clinical indices (Fig 1B–1I). In line with the previous reports, there was an inverse correlation between age and RTL (Fig 1B), although it was not statistically significant ($R^2 = 0.056$, $P = 0.12$), probably because of the relatively small sample size. There were no correlations between BMI, tumor diameter, random GH levels, nadir GH levels, or, IGF-I SDS, and RTL (Fig 1C and 1E–1H). Interestingly, there was a significant negative correlation between disease duration and RTL (Fig 1D; $R^2 = 0.210$, $P = 0.003$). After adjusting for

Table 1. Clinical characteristics of the acromegaly (Acro) and the non-functioning pituitary adenoma (NFPA) group.

	Acro (n = 46)	NFPA (n = 20)	*P*
Sex (male/female)	11/35	8/12	0.16
Age (years)	50.9 ± 12.0	56.2 ± 12.2	0.12
BMI	25.7 ± 3.0	25.3 ± 2.1	0.83
Smoking (%)	15.2	25.0	0.41
Diabetes (%)	19.4	7.8	0.33
HbA1c (%)	5.9 ± 0.77	5.4 ± 0.52	0.59
Hypertension (%)	34.2	38.5	0.78
Dyslipidemia (%)	26.8	35.3	0.52
Macroadenoma (%)	74.7	100	0.11
Tumor diameter (mm)	14.1 ± 5.7	24.8 ± 6.5	< 0.01**
Random GH (ng/mL)	12.1 [13.3]	0.2 [0.5]	< 0.01**
IGF-I SDS	6.6 ± 2.7	−0.2 ± 1.5	< 0.01**
PRL (ng/mL)	12.6 [13.8]	14.5 [16.1]	0.99
ACTH (pg/mL)	32.2 [20.8]	30.0 [25.1]	0.88
Cortisol (μg/dL)	12.6 ± 3.3	13.0 ± 3.5	0.68
TSH (μU/mL)	0.90 ± 0.55	1.25 ± 0.67	0.06
FT4 (ng/mL)	0.99 ± 0.22	0.96 ± 0.12	0.59
LH male (IU/mL)	2.1 [1.1]	3.1 [4.2]	0.29
LH female (IU/mL)	10.4 [11.7]	3.0 [10.7]	0.08
FSH male (IU/mL)	7.3 [7.3]	12.9 [8.8]	0.08
FSH female (IU/mL)	42.9 [55.1]	15.8 [36.2]	0.37
E2 (pg/mL)	7.1 [37.2]	0 [0]	0.12
T (ng/mL)	213.3 ± 78.2	280.8 ± 99.1	0.20

Data were compared by the χ^2 test, Fischer's exact test, Student's *t*-test, or Mann—Whitney test, as appropriate.

**$P < 0.01$.

RTL, relative telomere length; Acro, acromegaly; NFPA, non-functioning pituitary adenoma; E2, estradiol; T, testosterone. E2 and T were measured in female and male, respectively.

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the effect of age on RTL using multiple regression analysis, there remained a significant negative correlation between disease duration and RTL (β = −0.017, $P = 0.04$). RTL did not differ with the sex, smoking, or the presence of diabetes, hypertension, or dyslipidemia in patients with acromegaly (Fig 1I). Patients with hypertension were older than those without it (47.7 ± 12.3 v.s. 56.6 ± 8.6 years, $P = 0.02$), whereas there were no significant differences between these groups in age, sex, smoking habit, diabetes, and dyslipidemia. Since age is well known factor which influence telomere length, we re-analyzed with adjusting the effect of age using analysis of covariance (ANCOVA); however, there was no significant difference in telomere length between hypertensive and non-hypertensive patients (0.63 ± 0.28 v.s. 0.63 ± 0.26, $P = 0.82$) (S2 Fig).

Telomere length in human fibroblasts treated with GH or IGF-I

To explore the underlying mechanisms of telomere shortening in acromegaly, we analyzed the effect of GH or IGF-I treatment on telomere length in cultured human skin fibroblasts. We examined telomere length in logarithmic growth phase. Telomere length gradually shortened in each cell (Fig 2). Intriguingly, IGF-I-treated cells showed shorter telomeres than GH- or vehicle-treated cells at a PDL of 20 (Fig 2A and 2B). The telomere shortening rate, which is

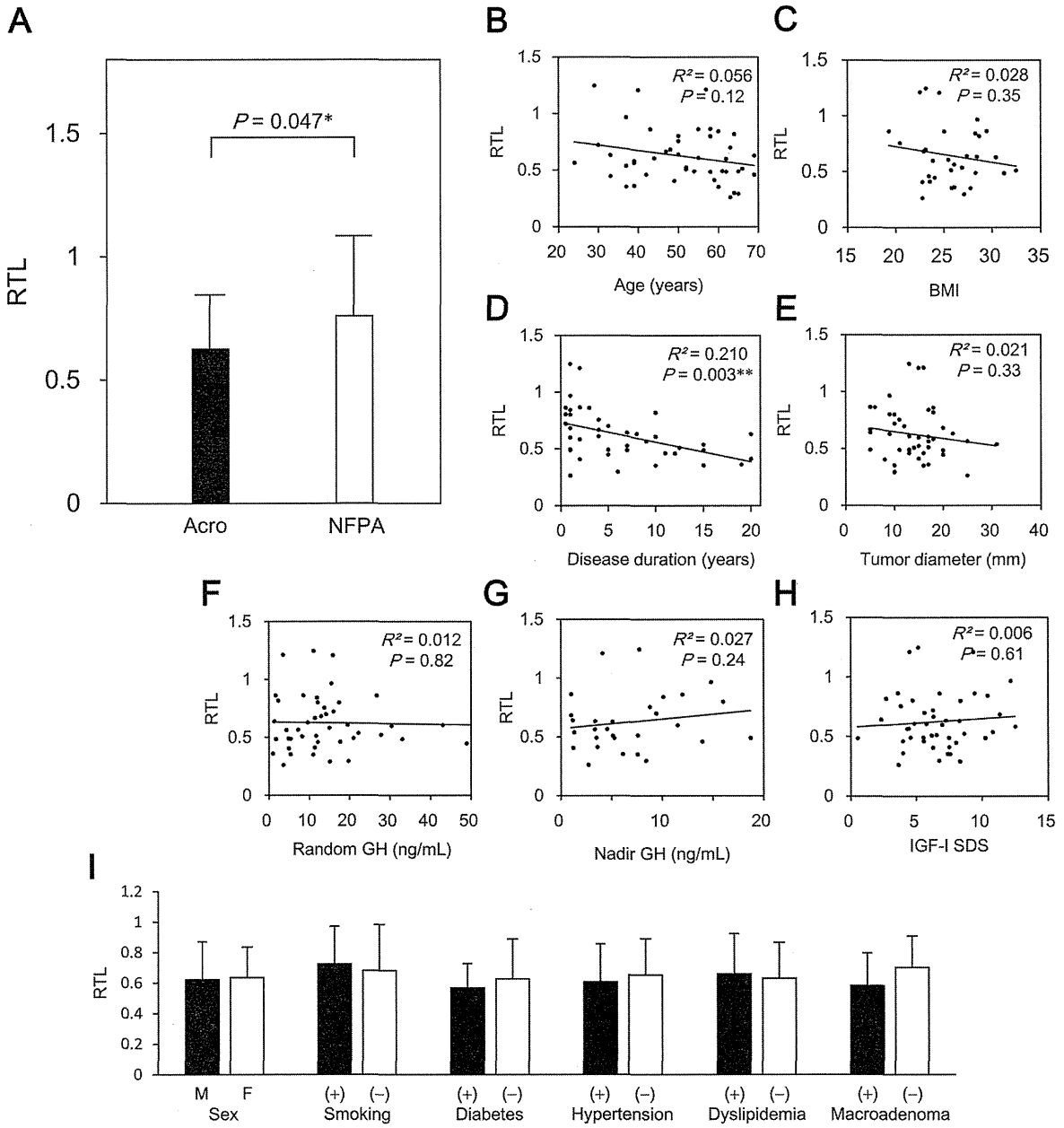

Fig 1. Telomere length in patients with acromegaly. A, Comparison of relative telomere length between patients with acromegaly and non-functioning pituitary adenoma. **B–H**, Correlations between relative telomere length and clinical indices (age, BMI, disease duration, tumor diameter, tandom GH, nadir GH during OGTT, and IGF-I SDS). **I**, Comparison of relative telomere length for the clinical indices (sex, hypertension, diabetes, dyslipidemia, macroadenoma) in patients with acromegaly. Relative telomere length was compared using Student's *t*-tests. Data are expressed as median with interquartile range. $*P < 0.05$, $**P < 0.01$. RTL, relative telomere length; Acro, acromegaly; NFPA, non-functioning pituitary adenoma.

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