No influence of parental origin of intact X chromosome and/or Y chromosome sequences on three-year height response to growth hormone therapy in Turner syndrome

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Purpose: Whether parental origin of the intact X chromosome and/or the presence of Y chromosome sequences (Yseq) play a role in three-year height response to growth hormone (GH) were investigated.

Methods: Paternal (Xp) or maternal (Xm) origin of X chromosome was assessed by microsatellite marker analysis and the presence of hidden Yseq was analyzed. The first-, second-, and third-year GH response was measured as a change in height z-score (Z_Ht) in Turner syndrome (TS) patients with 45,Xp (n=10), 45,Xm (n=15), and 45,X/46,X,+mar(Y) (Xm_Yseq) (n=8).

Results: The mean baseline Z_Ht did not differ according to Xp or Xm origin, however the mean baseline Z_Ht was higher in the Xm_Yseq group than in Xm group, after adjusting for bone age delay and midparental Z_Ht (P=0.04). There was no difference in the height response to GH between the 3 groups. The height response to GH decreased progressively each year (P<0.001), such that the third-year increase in Z_Ht was not significant. This third-year decrease in treatment response was unaffected by Xp, Xm, and Xm_Yseq groups. Increasing GH dosage from the second to third-year of treatment positively correlated with the increase in Z_Ht (P=0.017).

Conclusion: There was no evidence of X-linked imprinted genes and/or Yseq affecting height response to 3 years of GH therapy. Increasing GH dosages may help attenuate the decrease in third-year GH response in TS patients with 45,X and/or 46,X/+mar(Y).

Keywords: Turner syndrome, Growth hormone, X chromosome, Y chromosome, Genomic imprinting

Introduction

Growth failure is a problem for all girls with Turner syndrome (TS), with untreated individuals achieving an average adult stature 20 cm shorter than their peers1. Growth hormone (GH) treatment is now a standard of care for girls with TS2, which ameliorates growth failure and improves final adult height (FAH).

Cochrane database review in 20073 reported that GH doses between 0.3 to 0.375 mg/kg/wk increased short-term growth in girls with TS by approximately 3 cm (2 cm) in the first (second) year of treatment, and that mean FAH in 61 GH treated women was 148 cm compared to 141 cm in 43 untreated women. Despite GH therapy, girls treated with GH are still substantially shorter than other women as adults.

In the literature, factors affecting FAH include young age and bone age (BA) delay at the start...
of GH treatment, GH dose, duration of GH treatment, maternal X chromosome origin, target height, and good first-year height response to GH treatment. Growth response is known to decrease over the years of GH treatment. Good first-year response to GH therapy is important in determining FAH. However, in order to achieve FAH above the third percentile of the general population and height compatible with one’s genetic target height, good response to GH needs to be maintained. Increments in GH dose generate better short- and long-term response, thus identifying groups at risk of poor GH response and increasing doses accordingly is effective in terms of cost and safety.

Although one study reported that subjects with maternal X chromosome origin (Xm) had greater height gain during GH treatment than those with paternal X chromosome origin (Xp), the issue as to whether X-linked imprinting affects the GH response remains controversial. The phenotypic difference between Xp and Xm groups may reflect sexual dimorphism, because 46, XY males have only Xm. However, the role of Y chromosome sequences (Yseq) needs to be considered to explain the sexual dimorphism. To our knowledge, there has not yet been studies comparing GH responses in TS patients with Y chromosome sequences (Xm_Yseq) with 45,Xp or 45,Xm patients.

We analyzed TS patients with 45,X and/or 46,X.mar(Y) karyo-types to evaluate whether parental origin of the intact X chromosome and/or the presence of Yseq affects GH response during 3 years of therapy and plays a role in decreased GH response.

Materials and methods

This study was approved by the Seoul National University Hospital Ethics Committee. Informed consent was obtained from both the parents and children. All patients had confirmed karyotyping of 50 lymphocytes at the time of diagnosis of TS. Fifty-five TS patients (45,X, n=37; 45,X/46,X,+mar, n=18) were enrolled in genetic analyses to detect for Yseq and to determine parental origin of the intact X chromosome. Complete height data at the first, second and third year after start of GH therapy was available for analysis in a total of 33 patients (45,X, n=25; 45,X/46,X,+mar(Y), n=8). No patients took oral estrogen during these 3 years of GH therapy.

1. Analysis for detection of Y chromosome sequences

Polymerase chain reaction (PCR) using eight primers (PABY, SRY, ZFY, TSPY, DYS 270, DYS 280, DYS 209, and DYZ1) was performed to detect Yseq in TS subjects with cytogenetically proven (SRY positive) or unproven marker chromosome (45,X/46,X,+mar) and those subjects with 45,X karyotype. Female laboratory personnel who did not know the clinical characteristics of the enrolled TS patients performed all experiments to minimize the risk of producing false-positive results. Primers that amplify the CYP17A1 gene were used as an internal control for conventional PCR amplification of Y-linked markers to avoid false-negative results.

There were 8 patients enrolled in the study with the 45,X/46,X.mar(Y) karyotype, who either underwent gonadectomies before commencing GH treatment or did not show signs of virilisation throughout the duration of GH therapy. They had normal female external genitalia without signs of virilisation or spontaneous puberty throughout the GH treatment. All 8 patients were positive for PABY, SRY, ZFY, TSPY, and DYS 270 loci in analysis of the Y chromosome sequence, thus it was inferred that all patients contained the short arm and centromere (DYS 270) portion of the Y chromosome.

2. Assignment of parental origin of intact X chromosome

Confirmation of the parental origin of the intact X chromosome was analyzed by comparing the patient and parental DNA using nine highly polymorphic X chromosome microsatellites (DXS 1060, DXS 8051, DXS 1228, DXS 1214, DXS 986, DXS 990, DXS 1001, DXS 104, and DXS 8043), which were derived from the ABI PRISM Linkage Mapping Set MD-10 (Applied Biosystems Inc., Foster City, CA, USA). Highly polymorphic microsatellite markers were selected for their high percentage of heterozygosity (70.5%–86%), their allele number, and their location on both Xp and Xq based on a Korean reference. PCR amplification was performed under the following conditions: 95°C for 2 minutes, then 35 cycles at 95°C for 20 seconds, 58°C for 40 seconds, 72°C for 30 seconds, and 72°C for 45 minutes. None of the 45,X patients had hidden X fragments detected using X chromosome microsatellites. All 8 TS patients with 45,X/46,X.+mar(Y) were confirmed to have a Xm. Finally, those 25 patients with 45,X (Xp, n=10; Xm, n=15) and 8 patients with 45,X/46,X,+mar(Y) (Xm_Yseq) with complete height data were included in this study for analysis of height response to GH therapy.

3. The first-, second-, and third-year response of TS patients receiving GH therapy

The first-, second-, and third-year response to GH therapy was measured as a change in height z-score (Z_Ht) according to two different reference populations: TS specific height values as described by Lyon et al. (Z_Ht_Lyon) and the 2007 Korean reference height values (Z_Ht_07Kor). Given that there are no reliable Korean TS reference height values, the data of Lyon et al. was used for reference. Height (cm) was measured twice with a Harpenden stadiometer (Holtain Ltd., Crymlynch, Wales, UK) to one decimal place. The 2007 Korean reference height values were used for derivation of the midparental height z-score (Z_MPH) and body mass index z-score (Z_BMI). The proportion of poor responders to GH therapy, defined as those with a change in Z_Ht_Lyon < 0.1, was calculated. The percentage of patients below the third
percentile of the general population ($Z_{Ht\_07\_Kor} < -1.88$) was calculated. BA was assessed using the Greulich and Pyle method. Factors affecting growth response were investigated, including mean GH dose during each year of treatment (IU/kg/wk), difference in BA and chronologic age (BA delay), $Z_{MPH}$, and $Z_{Ht}$ at the start of GH (baseline) and after the first, second and third years of GH treatment. The first-, second-, and third-year change in $Z_{Ht}$ (first, second, and third year $\Delta Z_{Ht\_Lyon}$ and $\Delta Z_{Ht\_07\_Kor}$) was calculated, respectively.

4. Statistical analysis

Statistical analyses were performed using PASW Statistics ver. 18.0 (SPSS Inc., Chicago, IL, USA). All continuous variables are described as mean±standard deviation. The differences in the means of nonnormally distributed variables (GH dose) were tested by Kruskal-Wallis test. The other dependent variable was normally distributed for each 3 groups being compared. The differences in the means of continuous variables with homogenous variance between the 3 groups were tested by analysis of variance (ANOVA), and Welch’s ANOVA was used for other variable with heterogeneous variance. The difference in the means of variables within 2 subsets was analyzed using the Bonferroni method with $P$ set at 0.025. The chi-square test for trend was used to analyze the relationship between the 3 groups.

The significance of the difference in baseline $Z_{Ht}$ between the 3 groups (Xp, Xm, and Xm_Yseq group) was tested using analysis of covariance (ANCOVA), after controlling for the BA delay and $Z_{MPH}$. Heteroscedasticity-consistent standard errors were used to correct for heteroscedasticity in the ANCOVA. To test the influence of parental origin of intact X chromosome and/or Yseq across time points (baseline, 1 year, 2 years, and 3 years of GH therapy), we performed a repeated-measures analysis of variance with time as the within group factor and the 3 groups as the between group factor. In the between group analysis, the mean for each time points can be compared with the mean for each of the other time periods. To adjust for baseline $Z_{Ht\_Lyon}$, age at the start of GH, BA delay, mean GH dose for 3 years, $Z_{MPH}$, a series of repeated measures analysis of covariance models was used.

Possible factors significantly related to the third-year $\Delta Z_{Ht\_Lyon}$ in the simple linear regression were finally included in the multivariate regression model. Parental origin of intact X chromosome and/or Yseq (Xp/Xm/Xm_Yseq group), chronologic age (CA), $Z_{Ht\_Lyon}$, and BA delay after 2 years of GH therapy, $Z_{MPH}$, third-year GH dose, the change in GH dosage between the second and third-years of treatment (second year and third-year GH dose), first-year $\Delta Z_{Ht\_Lyon}$, and second-year $\Delta Z_{Ht\_Lyon}$ were selected as possible factors. Test for interaction was also used to analyze the modifying effect of the second-year $\Delta Z_{Ht\_Lyon}$ on the association between second year and third-year GH dose and the third-year $\Delta Z_{Ht\_Lyon}$, because the effect of the third-year $\Delta Z_{Ht\_Lyon}$ might vary depending on the second-year $\Delta Z_{Ht\_Lyon}$. Significance was defined as $P<0.05$.

Results

The mean baseline $Z_{Ht}$ at the start of GH therapy was $1.26±1.04$ ($Z_{Ht\_Lyon}$) and $-2.3±0.81$ ($Z_{Ht\_07\_Kor}$). The CA was $8.5±3.1$ years and the BA was $7.5±3.2$ years. The patients were started on GH treatments at a dose of $0.97±0.16$ U/kg/wk (range, $0.47–1.4$). The average GH dosage for all 3 years of treatment was $0.94±0.15$ U/kg/wk (range, $0.54–1.34$).

When baseline characteristics of the 3 groups (Xp, Xm, and Xm_Yseq group) were analyzed, there were no differences in baseline CA or BA. The significant difference in baseline $Z_{Ht\_Lyon}$ between the 3 groups was found ($0.75±0.61$ vs. $1.08±0.74$, $P=0.047$ by Welch’s ANOVA, Table 1). While there was no significant difference in baseline $Z_{Ht\_Lyon}$ between the Xp and Xm groups, the Xm_Yseq group had a higher baseline $Z_{Ht\_Lyon}$ than the Xm group, after adjusting for BA delay.

| Variable | Xp (n=10) | Xm (n=15) | Xm_Yseq (n=8) | $P$-value |
|----------|-----------|-----------|--------------|----------|
| CA at start GH (yr) | 7.2±3.0 | 9.0±3.4 | 9.2±2.6 | 0.344 |
| BA at start GH (yr) | 5.8±3.8 | 8.3±3.4 | 8.3±2.9 | 0.149 |
| BA delay (yr) | 1.39±1.12 | 0.73±1.58 | 0.84±0.60 | 0.427 |
| $Z_{MPH}$ | $-0.36±0.90$ | $-0.72±1.01$ | $-0.13±0.51$ | 0.245 |
| $Z_{Ht\_Lyon}$ | $0.75±0.61^a$ | $1.08±0.74^a$ | $2.21±1.39^a$ | 0.047 |
| $Z_{Ht\_07\_Kor}$ | $-2.5±0.61$ | $-2.44±0.71$ | $-1.75±1.03$ | 0.193 |
| $Z_{BMI\_07\_Kor}$ | $-0.17±1.57$ | $0.78±0.88$ | $0.81±0.96$ | 0.070 |

Values are presented as mean±standard deviation.

The differences in the means of continuous variables (baseline $Z_{Ht\_Lyon}$) with heterogenous variance between the 3 groups were tested by Welch’s analysis of variance (ANOVA).

Table 1. Baseline characteristics between TS patients with paternally derived X chromosome (Xp), maternally derived X chromosome (Xm), and those with 45,X/46,X,+mar(Y) (Xm_Yseq)
Z_Ht_Lyon significantly increased during 3 years of GH treatment ($P<0.001$) (Fig. 2A, Table 2). While Z_Ht_Lyon significantly increased by 0.69 in the first year ($P<0.001$) and by 0.38 in the second year of GH treatment ($P=0.003$), the third-year increase of 0.11 was not statistically significant ($P=0.99$). The average dose (IU/kg/wk) of GH was 0.97±0.16 (0.5–1.4), 0.92±0.17 (0.48–1.27), and 0.93±0.17 (0.64–1.35) during the first, second and third years of treatment, respectively. However, there was no difference between the 3 groups (Xp, Xm, and Xm_Yseq) as to the overall pattern of change in Z_Ht_Lyon with GH treatment ($P=0.536$) (Fig. 2A, Table 2). The differences in the mean Z_Ht_Lyon values during the 3 years (Xp vs. Xm_Yseq, $P=0.004$; Xm vs. Xm_Yseq, $P=0.013$) were no longer significant, after adjusting for baseline Z_Ht_Lyon, CA at the start of GH, BA delay, GH dose and Z_MPH ($P=0.715$) (Fig. 2A).

The proportion of poor responders ($\Delta Z_{Ht\_Lyon} < 0.1$) increased progressively from the first, second and third years of treatment by 9.1%, 27.3%, and 51.5% respectively (Fig. 2B). The percentage of short patients ($Z_{Ht\_07Kor} < –1.88$) declined successively from 69.7% at the time of GH commencement to 54.6% and 48.4% during the first and second years, but increased to 76.7% during the third year of treatment (Fig. 2C). These 25 short patients (76.7%) after 3 years of GH treatment included 8 (53.3%) of 15 patients who were consistently good responders for all 3 years, 12 of 13 patients (92.3%) who became poor responders in the third year of treatment, and all 5 patients (100%) who had remained poor responders for all 3 years ($P=0.011$, chi-square test for trend).

To identify groups at risk of poor GH response, factors affecting the third year response to GH (third year $\Delta Z_{Ht\_Lyon}$) were analyzed. In simple linear regression analysis, the third year $\Delta Z_{Ht\_Lyon}$ was negatively related to the second year $\Delta Z_{Ht\_Lyon}$ ($P=0.040$), and positively associated with the $\Delta$second and third year GH dose ($P=0.011$), respectively (Table 3). However, the origin of the intact X and/or the presence of Yseq did not have a significant effect on the third year $\Delta Z_{Ht\_Lyon}$ (Xm vs. Xp, $P=0.98$; Xm vs. Xm_Yseq, $P=0.69$) (Table 3). No significant correlation between the second year $\Delta Z_{Ht\_Lyon}$...
Table 2. Comparison of three-year height response to GH therapy between TS patients with paternally derived X chromosome (Xp), maternally derived X chromosome (Xm), and those with 45,X/46,X,+mar(Y) (Xm_Yseq)

| Variable                  | Xp (n=10) | Xm (n=15) | Xm_Yseq (n=8) | P-value |
|---------------------------|-----------|-----------|---------------|---------|
| GH dose IU/kg/wk (0–3 yr) | 0.91±0.15 | 0.95±0.18 | 0.95±0.07     | 0.794^a |
| ΔZ_Ht_Lyon (0–3 yr)       | 1.36±0.88 | 1.18±0.82 | 0.97±0.64     | 0.601^a |
| First-year response       |           |           |               |         |
| Z_Ht_Lyon                 | 0.14±0.87 | 1.86±0.78 | 2.75±1.18     | 0.015   |
| Z_Ht_07Kor                | −2.1±0.80 | −1.9±0.89 | −1.38±0.87    | 0.178   |
| Z_BMI_07Kor               | 0.51±1.35 | 0.61±0.99 | 0.51±0.83     | 0.932   |
| GH dose IU/kg/wk (0–1 yr) | 0.94±0.18 | 1.0±0.19  | 0.95±0.06     | 0.412^a |
| ΔZ_Ht_Lyon (0–1 yr)       | 0.66±0.41 | 0.78±0.35 | 0.54±0.70     | 0.488   |
| HV (cm/yr)                | 7.3±1.6   | 8.2±1.9   | 7.7±1.7       | 0.389   |
| Second-year response      |           |           |               |         |
| Z_Ht_Lyon                 | 2.0±0.89  | 2.18±0.85 | 3.0±0.90      | 0.054   |
| Z_Ht_07Kor                | −1.98±0.92| −1.84±0.94| −1.25±0.75    | 0.311   |
| Z_BMI_07Kor               | 0.04±1.42 | 0.75±1.10 | 0.53±0.87     | 0.122   |
| GH dose IU/kg/wk (1–2 yr) | 0.87±0.18 | 0.93±0.19 | 0.93±0.14     | 0.526^a |
| ΔZ_Ht_Lyon (1–2 yr)       | 0.6±0.62  | 0.31±0.66 | 0.25±0.84     | 0.484   |
| HV (cm/yr)                | 6.8±1.7   | 6.0±2.1   | 5.9±0.8       | 0.486   |
| Third-year response       |           |           |               |         |
| Z_Ht_Lyon                 | 2.11±0.90 | 2.26±0.95 | 3.18±1.08     | 0.056   |
| Z_Ht_07Kor                | −1.81±0.87| −1.81±0.66| −1.04±0.83    | 0.122   |
| Z_BMI_07Kor               | −0.14±1.43| 0.81±0.07 | 0.45±0.73     | 0.172   |
| GH dose IU/kg/wk (2–3 yr) | 0.93±0.16 | 0.93±0.21 | 0.93±0.14     | 0.910^a |
| ΔZ_Ht_Lyon (2–3 yr)       | 0.09±0.44 | 0.09±0.57 | 0.18±0.55     | 0.912   |
| HV (cm/yr)                | 5.7±0.9   | 5.5±1.4   | 5.6±1.3       | 0.885   |

Values are presented as mean±standard deviation. The differences in the means of nonnormally distributed variables (GH dose IU/kg/wk (0–3 yr), GH dose IU/kg/wk (0–1 yr), GH dose IU/kg/wk (1–2 yr), and GH dose IU/kg/wk (2–3 yr) were tested by Kruskal-Wallis test^a. Other variables between the 3 groups were normally distributed. Analysis of variance was used for other variables with homogeneous variance. The difference in the means of variables within 2 subsets was analyzed using the Bonferroni method with P set at 0.025 (^aP<0.025).

GH, growth hormone; Z_Ht_Lyon, height z-score according to Turner syndrome specific height reference by Lyon et al.^12; Z_Ht_07Kor, height z-score according to the 2007 Korean reference height values; Z_BMI, body mass index z-score; ΔZ_Ht_Lyon, change in Z_Ht_Lyon; and HV, height velocity.

The mean baseline Z_Ht did not differ according to Xp or Xm origin, however the mean baseline Z_Ht was higher in the Xm_Yseq group than in Xm group, after adjusting for BA delay and Z_MPH. When this difference in baseline Z_Ht was accounted for, there was no difference between the 3 groups in the response to 3 years of GH therapy. The height response to GH therapy decreased progressively each year, such that the increase in Z_Ht was not significant during the third year of therapy. This third-year decrease in treatment response was unaffected by parental origin of X chromosome and/or presence of Yseq. The response...
than in pseudoautosomal regions. Previous studies that compared phenotypic differences in 3 
groups may reflect sexual dimorphism. There have not been 
differences in baseline $Z_{HT}$ of Xp and Xm groups as well as no 
sequences (pure Xp or Xm) to exclude the effect of different 
include only 45,X patients without hidden X or Y chromosome 
confirm or exclude X chromosomal imprinting. In our study, we 
replicated convincingly, and additional studies are needed to 
effect in patients with TS or Klinefelter syndrome has not been 
Hamelin et al. 
the intact X chromosome to the response to GH treatment. 
132 www.e-apem.org 
by Lyon et al. 
suggested that a stature-determining critical region can 
be mapped between Xp22.1 and Xp11.2, which is located 
and BA; Xp, paternally derived X chromosome; Xm, maternally 
derived X chromosome; Xm_Yseq, 45,X/46,X,+mar(Y); $Z_{MPH}$, 
midparental height z-score; First- and second-year Δ$Z_{HT-Lyon}$, 
and first- and second-year change in $Z_{HT-Lyon}$ (first- and second-
year height response).

to GH treatment during the third year was positively correlated 
with the increase in GH dose from the second to third year.

In contrast to several autosomal genes identified in humans 
and X-linked imprinted genes in mice, an X-linked imprinted 
gene has not been identified in humans. Phenotypic differences 
between TS patients with Xm and those with Xp suggest the 
presence of X-linked imprinted genes in humans. Zinn et 
S.E., Standard error; GH, growth hormone; $Z_{HT-Lyon}$, height 
z-score according to Turner syndrome specific height reference 
by Lyon et al. 
; BA, bone age; BA delay, difference between CA 
and BA; Xp, paternally derived X chromosome; Xm, maternally 
derived X chromosome; Xm_Yseq, 45,X/46,X,+mar(Y); $Z_{MPH}$, 
midparental height z-score; First- and second-year Δ$Z_{HT-Lyon}$, 
and first- and second-year change in $Z_{HT-Lyon}$ (first- and second-
year height response).

Table 3. Simple linear regression analysis with third-year height 
response as the dependent variable

| Variable | β  | S.E | t-test | P-value |
|----------|----|-----|--------|---------|
| Age at 2 years after GH | -0.026 | 0.030 | -0.851 | 0.401 |
| $Z_{HT-Lyon}$ at 2 years after GH | -0.041 | 0.100 | -0.411 | 0.684 |
| BA delay at 2 years after GH | 0.022 | 0.071 | 0.306 | 0.762 |
| Xp group (vs. Xm group) | 0.006 | 0.138 | 0.408 | 0.697 |
| Xm_Yseq group (vs. Xm group) | 0.096 | 0.235 | 0.408 | 0.686 |
| $Z_{MPH}$ | 0.045 | 0.104 | 0.432 | 0.669 |
| Third-year GH dosage | 0.558 | 0.529 | 1.054 | 0.300 |
| The change in GH dose from 
the second to third years | 4.570 | 1.690 | 2.750 | 0.011 |
| First-year Δ$Z_{HT-Lyon}$ | -0.070 | 0.200 | -0.352 | 0.727 |
| Second-year Δ$Z_{HT-Lyon}$ | -0.271 | 0.127 | -2.139 | 0.040 |

The phenotypic difference between Xp, Xm and Xm_Yseq 
groups may reflect sexual dimorphism. There have not been 
previous studies that compared phenotypic differences in 3 
groups of TS patients. In our study, changes in height of the 3 
groups without missing data during 3 years of GH therapy were 
analyzed. Although Xm_Yseq patients had greater baseline $Z_{HT}$ 
than Xm and Xp patients, there was no significant difference 
in the response to GH therapy. Despite the fact that height is 
a multifactorial trait affected by genetic and environmental 
factors, the greater baseline height of Xm_Yseq over Xm 
suggests that the Y chromosome harbors genes that affect 
height. This hypothesis is also supported by the observations 
that XY females are generally taller than XX females, that XY 
males are taller than XX males\(^{\text{19}}\), and that patients with pure 
XX or XY gonadal dysgenesis show height differences despite 
having gonadal steroid deficiency\(^{\text{20}}\). Correlations between 
breakpoints on Yq and the height of respective patients assigned 
the growth controlling gene on the Y chromosome (GCY) 
locus to the pericentromeric region of Yq11\(^{\text{21}}\). Kirsch et al.\(^{\text{22}}\) 
confirmed the proximal interval between markers DYZ3 and 
DYS11 as the GCY critical interval on Yq using detailed 
Fluorescence in situ hybridization and high density sequence-
tagged sites (STS) analysis. DYS 11 is located between DYS 
270 and DYS 280, which STS were included in our analysis. 
The marker chromosome of the Xm_Yseq patients in this 
study contained the Y chromosome short arm (Yp) and the 
DYS 270 locus, making it likely that the GCY critical interval 
is also included. Previous reports indicate GH-deficient men 
are more responsive to GH therapy than are women\(^{\text{23}}\). Thangavel 
and Shapiro\(^{\text{24}}\) proposed that the cellular actions of GH, 

When the response to GH therapy during the third year was 
assessed, there was no significant increase in $Z_{HT}$ during this 
period with more than 50% of patients being poor responders. 
The percentage of short patients smaller than the third 
percentile ($Z_{HT-07Kor<–1.88}$) increased from 69.6% at the 
commencement of GH therapy to 76.7% during the third year. 
Starting in the year 2000, the insurance coverage guidelines of 
the Korean Ministry of Health and Welfare recommended GH 
doses of 1.0 U/kg/wk up to a height of 150 cm. An additional 
provision to increase this dosage to a maximum of 1.4 U/kg/ 
wk was added in the year 2010 for those patients with poor 
response up to a height of 150 cm. The patients in this study 
received GH according to the guidelines before 2010, so there 
was a restriction in the range of GH increments. Although there 
was a general tendency to increase the GH dosage with poor 
response during the previous year, the incremental range was 
too small to show a significant correlation between previous 
response to GH therapy and next year GH dose increment. The 
response to GH therapy progressively declined so that there was 
no significant response during the third year of therapy. The 
decline in response did not differ between Xp, Xm, and Xm_Yseq 
groups. The change in GH dosage from the second to third 
year of treatment was the only significant predictor influencing
the third-year growth response. A recent study proposed that the dose increments in the second and third years of treatment in patients with poor response during the previous year attenuated the normal decline in GH response \(^9\). A study of step-up dosage regimes published by van Pareren et al.\(^9\) showed that dose increments had a significant effect on final height, although the effect was discernible only after 4–7 years. GH dosage needs to be increased at the third year without regard to the response during the previous year or parental origin of X chromosome and/or Yseq. This study included only those TS patients with pure 45,X and 45.X/46.X.+mar(Y) karyotypes who had complete height data throughout 3 years of GH therapy and is therefore limited by the small sample size. Additionally, the effect of GH therapy on FAH was not included in this study. Once patients reached a height of 150 cm, Korean national insurance guidelines did not provide GH coverage. GH treatments were usually discontinued even though FAH was not attained, making analysis of the GH response in determining FAH difficult. Despite limitations, this is the first study to assess three year GH response in TS patients including pure Xp, pure Xm homogenous subjects and in those with Xm_Yseq karyotypes.

In conclusion, there was no evidence of X-linked imprinted genes and/or Yseq affecting height response to three years of GH therapy. Without regard to the parental origin of intact X chromosome and/or presence of Yseq and GH response during the previous year, increasing GH dosages may help attenuate the decrease of the third-year response to GH therapy in TS patients with 45.X and/or 46.X+/mar(Y).

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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