Abstract. The Growject® database on human GH treatment in Turner syndrome was analyzed in the Turner Syndrome Research Collaboration, and the relationships of the frequencies of spontaneous breast development and spontaneous menarche with karyotype and GH treatment were investigated. One hundred and three cases started GH treatment with 0.5 IU/kg/ week (0.5 IU group), and their
dose was increased to 0.35 mg/kg/wk midway through the treatment course. Another 109 cases started GH at a dose of 0.35 mg/kg/wk (0.35 mg group). Spontaneous breast development was observed in 77 (36.3%) of the 212 patients, and spontaneous menarche occurred in 31 patients (14.6%). The frequency of spontaneous breast development was significantly lower in patients with the 45,X karyotype and significantly higher in patients with a structural abnormality of the second X chromosome. The frequency of spontaneous menarche was significantly higher in patients with mosaicism characterized by X monosomy and a cellular line with no structural abnormality of the X chromosome. No significant differences in frequencies of spontaneous breast development and spontaneous menarche were observed between the two dose groups, indicating that GH treatment does not increase the frequency of spontaneous puberty.

**Key words:** Turner syndrome, GH treatment, spontaneous breast development, spontaneous menarche, chromosome

---

**Introduction**

Turner syndrome (TS) is a congenital disease characterized by infantilism (genital hypoplasia, short stature), congenital webbed neck, and cubitus valgus deformity first described by Henry Turner (1) in 1938 in the United States. In Europe, similar cases were reported by Ullrich (2) in 1930; thus TS is also called Ullrich-Turner syndrome.

Although hypogonadism is a characteristic of TS, Ferguson-Smith reported in 1965 that 7 out of 83 (8.4%) TS patients with a karyotype of 45,X, and 7 out of 34 (20.6%) patients with a karyotype of 45,X/46,XX had spontaneous menstruation (3). In Japan, Hibi (4) first reported the frequency of spontaneous puberty as 21.3% in the First International Turner Syndrome Symposium at the 50th anniversary of the report by Turner.

Several papers have reported the relation between the frequency of spontaneous puberty and chromosome karyotypes and showed a low frequency of X-monosomy and high frequency of X-monosomy mosaicism and cellular lines without structural abnormalities of the second X.

The increase in IGF-I induced by GH treatment indicates facilitation of ovarian function (5, 6), and it was thus presumed that GH treatment increased the frequency of spontaneous puberty in TS patients.

In this study, we confirmed the relationships of the frequencies of spontaneous breast development and spontaneous menarche with karyotype and GH treatment described in previous reports.

**Subjects and Methods**

In Japan, TS patients with GH deficiency (GHD) received GH treatment for the first time in 1991; however, at that time, the patients were only treated with a dose of 0.5 IU/kg/wk (0.167 mg/kg/wk). In November 1999, 1.0 IU/kg/week of Growject® (Japan Chemical Research Co., Ltd., Hyogo, Japan, currently JCR Pharmaceuticals Co., Ltd.) was approved as the standard in all TS cases, not only for those with GHD. In accordance with the change in units from IU to mg, a dose of 0.35 mg/kg/wk has been the standard since 2001.

To investigate the efficacy and safety of treatment with Growject®, the Turner Syndrome Research Collaboration (TRC) was established in 2000 to conduct post-marketing surveys and analyze data.

Among 249 cases enrolled in the TRC between 2000 and 2008, 212 cases 9 yr of age or
older in 2008 were investigated. They all received GH at a dose of 0.35 mg/kg/wk till 2007. One hundred and three cases started GH treatment with 0.5 IU/kg/wk (0.5 IU group) at a mean age of 7.9 ± 3.1 yr (1.7–14.9 yr) before 2000, and the dose was increased to 0.35 mg/kg/wk midway through the treatment course at a mean age of 11.9 ± 3.3 yr (2.9–18.9 yr) after a mean duration of 4.1 ± 3.0 yr at 0.5 IU/kg/wk of GH. Another 109 cases started GH at a dose of 0.35 mg/kg/wk (0.35 mg group) after 2001, and their mean age at the start of GH treatment was 9.5 ± 3.4 yr (1.3–22.7 yr). Onset of puberty was defined as breast development (Tanner stage 2) as determined by the attending physician, and menarche was defined as the first genital bleeding.

This study was conducted with the approval of the ethics committees of the individual institutes and written informed consent of the patients and/or their parents was obtained.

Data are expressed as the mean ± SD. The χ² test was used in frequency comparisons among different karyotype groups. Frequency comparisons of the development of secondary sexual characteristics between GH doses were performed using the χ² test at each age. P < 0.05 was considered significant.

**Results**

In 212 patients 9 yr of age or older, spontaneous breast development was observed in 42 (40.7%) of 103 cases in the 0.5 IU group and 35 (32.1%) of 109 cases in the 0.35 mg group. No significant difference was observed in the proportion of patients showing spontaneous breast development between the two groups. Overall, breast development was observed in 36.3% of patients (Table 1). However, 13 cases (31.0%) in the 0.5 IU group and 8 cases (22.9%) in the 0.35 mg group did not mature puberty and required estrogen replacement therapy.

As shown in Tables 2 and 3, patients were divided into five groups according to the chromosomal karyotype as follows: Group 1 consisted of 64 cases (30.2%) with 45,X; Group 2 consisted of 23 cases (10.8%) with a structural abnormality in the second X in a cell line [46,X,i(X), 46,X,del(X), 46,X,r(X), etc.]; Group 3 consisted of 28 cases (13.2%) with a 45,X

| Table 1. Frequencies of spontaneous breast development and spontaneous menarche |
|-----------------------------|-----------------|---------------------|
| N  | Breast development | Menarche |
|-----------------------------|-----------------|---------------------|
| 0.5 IU group | 103 | 42 (40.7%) | 17 (16.5%) |
| 0.35 mg group | 109 | 35 (32.1%) | 14 (12.8%) |
| Total | 212 | 77 (36.3%) | 31 (14.6%) |

| Table 2. Breast development and karyotypes |
|-----------------------------|-----------------|---------------------|
| Group | Chromosome | N  | Observed cases | Expected cases | P |
|-----------------------------|-----------------|---------------------|
| 1 Monosomy X | 64 | 13 (20.3%) | 23.2 | < 0.05 |
| 2 Structural abnormality of second X | 23 | 15 (65.3%) | 8.4 | < 0.05 |
| 3 X-monosomy/celluar line without structural abnormalities of second X | 28 | 17 (60.7%) | 12.3 | NS |
| 4 X-monosomy/celluar line with structural abnormalities of second X | 88 | 28 (31.8%) | 32.0 | NS |
| 5 Other abnormalities | 9 | 4 (44.4%) | 1.1 | NS |
mosaic and without a structural abnormality of the X chromosome in the second cell line (45,X/46,XX, 45,X/47,XXX, 45,X/46,XX, 47XXX, etc.); Group 4 consisted of 88 cases (41.5%) with a 45,X mosaic and a structural abnormality of the second X chromosome in the second cell line [45,X/46,X,i(X), 45,X/46,X,del(X), 45,X/46,X,r(X), etc.]; and Group 5 consisted of other 9 cases (4.2%) with 45,X/46,XY, 45,X/47,XXY, 45,X,inv(9p+q-)/45,X,+mar, 46,X,+mar/48,XXX,+mar, etc.

Table 2 shows the frequencies of spontaneous breast development in the individual groups. When the expected number and actual number in each group were compared using the \(\chi^2\) test, the frequency of spontaneous breast development was significantly lower in Group 1 (\(P < 0.05\)) and significantly higher in Group 2 (\(P < 0.05\)). Although Group 3 showed a higher actual number than the expected number, the difference did not reach statistical significance.

Table 3 shows the frequencies of spontaneous menarche in the individual groups. The frequency of spontaneous menarche was significantly higher in Group 3 (\(P < 0.01\)). In Group 3, the karyotypes associated with spontaneous menarche were 45,X/46,XX (6 cases), 45/X/47,XXX (6 cases) and 45,X/46,XX/47,XXX (1 case). In mosaic with 46,XX and 47,XXX, a high frequency of spontaneous menarche similar to that of 45,X/46,XX was observed.

Figure 1 shows the age-specific frequencies of spontaneous breast development. Breast development started at approximately 9 yr of age and reached a plateau at approximately 17 yr of age, after exceeding 40%. No significant difference was observed between the two dose groups.

Spontaneous menarche occurred in 17 (16.5\%) cases in the 0.5 IU group and 14 (12.8\%) cases in the 0.35 mg group. No significant difference was observed between the groups. Overall, spontaneous menarche occurred in 14.6\% of patients. However, 4 cases (23.5\%) in the 0.5 IU group and 2 cases (14.3\%) in the 0.35 mg group showed cessation of menstruation requiring cyclic estrogen-progesterone therapy.

Figure 2 shows the age-specific frequencies of spontaneous menarche. Spontaneous breast development occurred in all the cases. Menarche occurred at approximately 11 yr of age and plateaued at the age of 14, after exceeding 15\%. No significant difference was observed in the frequency between the two dose groups.

Discussion

Although gonadal function in TS is impaired in many cases and estrogen replacement therapy is required, there are some TS patients who undergo spontaneous puberty, become pregnant and then have babies. In our study, we investigated the gonadal function of 212 TS patients who were 9 years old or older and receiving GH treatment and found that spontaneous breast development occurred in 77 patients (36.3\%) and that spontaneous menarche
Spontaneous puberty in Turner syndrome occurred in 31 patients (14.6%).

Regarding the correlations between karyotype and spontaneous menarche in TS, Ferguson-Smith already reported in 1965 that spontaneous menarche occurred at high frequency in mosaic with 45,X/46,XX and at low frequency in 45,X (3). Overall frequencies of spontaneous menarche were reported to be between approximately 15% and 35% including our study (Table 4) (7–10). The frequencies

Fig. 1. Age-specific frequency of spontaneous breast development in Turner syndrome.

Fig. 2. Age-specific frequency of spontaneous menarche in Turner syndrome.
Spontaneous menarche occurred in 5% to 10% of cases of 45,X karyotype and approximately 40% of cases of mosaic with 45,X and cellular lines without structural abnormalities of the second X. The frequency of spontaneous menarche is almost constant in these two karyotypes. Our study demonstrated that both the 45,X/46,XX and 45,X/47,XXX karyotypes are equally associated with the preservation of ovarian function.

Regarding the correlation between karyotype and spontaneous breast development, overall frequencies of spontaneous breast development were reported to be between approximately 20% and 50% (Table 4). These frequencies also depended on the karyotype distribution. Spontaneous breast development occurred in between 0% and 20% of cases of 45,X karyotype and between 60% and 100% of cases of mosaic with 45,X and cellular lines without structural abnormalities of the second X. The wide range of frequencies may be explained by the difference in timing of diagnosis and difficulty in defining breast development in obese girls.

The abovementioned studies (7–10) also describe cases in which estrogen replacement was required because full sexual maturation was not reached, despite the spontaneous onset of puberty. These findings indicate that there are many cases of incomplete ovarian function.

Regarding the relation between GH treatment and the frequency of spontaneous puberty, Hibi (11) analyzed patients with TS receiving GH treatment in Japan and showed that the cumulative frequency of spontaneous

**Table 4. Summary of the literature including the present study**

|                | Breast development | Menarche                  |
|----------------|--------------------|---------------------------|
| Ferguson-Smith (1965) (3) |                    | 45,X: 8.4% (7/83)         |
|                 |                    | 45,X/46,XX: 20.6% (7/34)  |
| Lippe (1993) (7) | 21% (29/141)       |                           |
|                 | 45,X: 8.9% (7/79)  |                           |
|                 | 45,X/46,XX: 80% (8/10) |                       |
| Carpini (2012) (8) | Early diagnosis (< 10 yr) | 18.8% (6/32)          |
|                 | 50% (16/32)        |                           |
|                 | 45,X: 0% (0/12)    |                           |
|                 | 45,X/46,XX: 100% (10/10) |                     |
|                 | Late diagnosis (≥13 yr) | 28.6% (18/63)      |
| Hagen (2010) (9) | 34% (15/44)        |                           |
|                 | 45,X: 6% (1/29)    |                           |
|                 | 45,X/46,XX: 89% (8/9) |                       |
| Pasquino (1997) (10) | 33.5% (175/522)    | 16.1% (84/522)           |
|                 | 45,X: 22.4% (61/272) |                           |
|                 | 45,X/no structural abnormality: |               |
|                 | 58.0% (40/69)      | 45,X/no structural abnormality: | 40.6% (28/69) |
| Present study   | 36.3% (77/212)     | 14.6% (31/212)           |
|                 | 45,X: 20.3% (13/64) |                           |
|                 | 45,X/no structural abnormality: |               |
|                 | 58.8% (20/34)      | 45,X/no structural abnormality: | 38.2% (13/34) |
breast development was 21.3% among 75 patients aged 15 or older, which was similar to that in patients who did not receive GH treatment in his previous study (4). The lower rate of spontaneous breast development may be explained by the late diagnosis. Considering an Italian report (10) and our current study, which found that the frequencies of spontaneous breast development and spontaneous menarche did not differ when the GH dose was doubled, it is concluded that GH treatment does not affect the frequency of spontaneous puberty in TS.

In conclusion, among the Japanese TS patients with GH treatment enrolled in the TRC, the frequencies of spontaneous breast development and spontaneous menarche were 36.3% and 14.6%, respectively, which are similar to those reported in Europe and the United States. Both the 45,X/46,XX and 45,X/47,XXX karyotypes were associated with similar preservation of ovarian function, as determined by the frequency of spontaneous menarche. GH doses did not affect the frequency of spontaneous puberty, and GH treatment did not increase the frequency of spontaneous puberty.

**Acknowledgments**

We thank the attending physicians for participating in the TRC and registering TS cases.

**References**

1. Turner HH. A syndrome of infantilism, congenital webbed neck, and cubitus valgus. Endocrinology 1938;23: 566–74. [CrossRef]
2. Ullrich O. Über typische Kombinationsbilder mulipler Abartung. Z Kinderheilkd 1930;49: 271–6. [CrossRef]
3. Ferguson-Smith MA. Karyotype-phenotype correlations in gonadal dysgenesis and their bearing on the pathogenesis of malformations. J Med Genet 1965;2: 142–55. [Medline] [CrossRef]
4. Hibi I, Tanae A. Final height in Turner syndrome with and without gonadal function. In: Rosenfeld R, Grumbach MM, editors. Turner syndrome. New York and Basel: Marcel Dekker: 1990. p. 163–74.
5. Adashi EY, Resnick CE, D’Ercole AJ, Svoboda ME, Van Wyk JJ. Insulin-like growth factors as intraovarian regulators of granulosa cell growth and function. Endocr Rev 1985;6: 400–20. [Medline] [CrossRef]
6. Davoren JB, Hsueh AJ. Growth hormone increases ovarian levels of immunoreactive somatomedin C/insulin-like growth factor I in vivo. Endocrinology 1986;118: 888–90. [Medline] [CrossRef]
7. Lippe B, Westra SJ, Boechat MI. Ovarian function in Turner syndrome: recognizing the spectrum. In: Hibi I, Takano K, editors. Basic and clinical approach to Turner syndrome. London, New York, Tokyo: Excerpta Medica: 1993. p. 117–22.
8. Carpini S, Carvalho AB, Guerra-Júnior G, Baptista MTM, Lemos-Marini SHV, Maciel-Guerra AT. Spontaneous puberty in girls with early diagnosis of Turner syndrome. Arq Bras Endocrinol Metab 2012;56: 653–7. [Medline] [CrossRef]
9. Hagen CP, Main KM, Kjaergaard S, Juul A. FSH, LH, inhibin B and estradiol levels in Turner syndrome depend on age and karyotype: longitudinal study of 70 Turner girls with or without spontaneous puberty. Hum Reprod 2010;25: 3134–41. [Medline] [CrossRef]
10. Pasquino AM, Passeri F, Pucarelli I, Segni M, Municchi G. Italian Study Group for Turner’s Syndrome Spontaneous pubertal development in Turner’s syndrome. J Clin Endocrinol Metab 1997;82: 1810–3. [Medline]
11. Hibi I, Tanae A, Tanaka T, Yoshizawa A, Miki Y, Ito J. Spontaneous puberty in Turner syndrome: its incidence, influence on final height and endocrinological feature. In: Ranke MB, Rosenfeld R, editors. Turner’s syndrome and growth-promoting therapies. Amsterdam, New York, Oxford: Excerpta Medica: 1991. p. 75–81.