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Growth Hormone and Gonadotropin-Releasing Hormone Analog Therapy in Haploinsufficiency of SHOX

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Abstract. We report on GH (0.5 IU or 0.17 mg/kg/week) and GnRH analog (GnRHa, 60 μg/kg, every 4 weeks) therapy in SHOX haploinsufficiency. Case 1 was a 46,XY boy with microdeletion of the Y chromosomal pseudoautosomal region. At 7 years of age, he exhibited short stature (−3.9 SD) with a reduced growth rate (3.8 cm/year), short 4th metacarpals, and mild Madelung deformity. GH therapy resulted in a marked increase in height velocity (10.7 cm/year in the first year). Case 2 was a 46,XX girl with a heterozygous nonsense mutation of SHOX (C674T). At 6 years of age, she presented with short stature (−3.3 SD) with a low height velocity (4.0 cm/year). GH therapy caused a moderate increase in height velocity (6.6 cm/year in the first year and 6.0 cm/year in the second year) before puberty. Because of breast development, she received GnRHa from 9 8/12 years of age. At 10 10/12 years of age, she had mild shortening and borderline curvature of radius. Case 3 was a girl with a 46,X,der(X)t(X;2)(p22.3;p21) karyotype. She was treated with GH from 6 to 14 years of age, and also with GnRHa from 12 to 15 years of age. Her height remained around mean −4 SD, with no discernible alteration of height velocity. At 17 years of age, she had short stature (−4.1 SD), bilateral cubitus valgus, Madelung deformity, and full breast development. The results suggest that GH therapy may have variable statural effects in SHOX haploinsufficiency as in most disorders including Turner syndrome, and that GnRHa therapy after pubertal entry may be insufficient to prevent the development of skeletal lesions such as Madelung deformity.

Key words: SHOX, Short stature, Madelung deformity, GH therapy, GnRH analog

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SHOX (short stature homeobox containing gene) cloned from the short arm pseudoautosomal region (PAR1) of the human sex chromosomes is the first gene that has been shown to be relevant to the development of specific features in Turner syndrome [1]. Clinical studies in patients with SHOX mutations or pseudoautosomal microdeletions involving SHOX have demonstrated that SHOX haploinsufficiency causes not only short stature but also Turner skeletal features such as short metacarpals, cubitus valgus, and Madelung deformity [1–5]. Since skeletal features are more severe in females than in males and become obvious with puberty in SHOX deficiency, it has been suggested that gonadal estrogens exert a maturational effect on skeletal tissues that are susceptible to unbalanced premature fusion of growth plates because of SHOX haploinsufficiency, facilitating the development of skeletal lesions and resultant growth deficiency in a female-dominant and pubertal tempo-influenced fashion [4].

For SHOX haploinsufficiency, there may be two therapeutic implications. First, since GH therapy is

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effective in Turner syndrome despite the absence of GH deficiency [6], it may also be advantageous in SHOX haploinsufficiency. In support of this, Binder *et al.* [7] have reported a beneficial effect of 1-year GH therapy (1.0 IU or 0.33 mg/kg/week) in prepubertal sibs with a heterozygous nonsense mutation of SHOX (C674T) and apparently low-normal or subnormal endogenous GH secretion, with an increase in height velocity from −2.0 SD to +5.4 SD (9.4 cm/year) for the boy and from −2.3 SD to +3.9 SD (9.5 cm/year) for the girl. Shanske *et al.* [8] have also described a similar 1-year GH effect in a prepubertal boy with 45,X,dic(Yp;13p) missing SHOX and apparently normal endogenous GH secretion, with an increase in height velocity from 2.8 cm/year to 9.4 cm/year. Second, since GnRH analog (GnRHa) therapy can suppress gonadal estrogen production, it may serve to prevent the development of skeletal features. To our knowledge, however, there are only two reports describing GH therapy in patients with proven SHOX haploinsufficiency [7, 8], and there has been no report documenting GnRHa therapy in such patients. In this paper, we report our experience on GH and GnRHa therapy in SHOX haploinsufficiency.

Case reports

Case 1

This boy was born at 39 weeks of gestation, with a length of 44 cm (−3.1 SD) and a weight of 2.35 kg (−2.1 SD). At 7 1/12 years of age, he was seen because of short stature. The height was 100.7 cm (−3.9 SD) and the weight 15.7 kg (−2.0 SD) (Fig. 1). He exhibited mild short 4th metacarpals and mesomelic appearance but had no cubitus valgus. Psychomotor development was normal. Bone age (BA) was assessed as 6 years by the TW-2 method standardized for Japanese [11]. Radiographs of the hands and forearms indicated bilateral short 4th metacarpals and mild radial curvature suggestive of mild Madelung deformity (Fig. 2). The peak serum GH level was 5.6 ng/ml after arginine stimulation and 11.7 ng/ml after insulin stimulation, and GH secretion measured for 24-hour collected urine was 29.5, 48.2, and 39.2 ng/g·creatinine for three consecutive days. Serum IGF-I was 120 ng/ml (age-matched normal range, 65–340 ng/ml) and IGFBP-3 2.08 μg/ml (0.63–4.45 μg/ml). Other laboratory and endocrine studies were normal. His karyotype was 46, XY in 30 lymphocytes analyzed. Fluorescence in situ hybridization (FISH) analysis was carried out with probes for Xp/Yp telomere, SHOX, DXYS59,

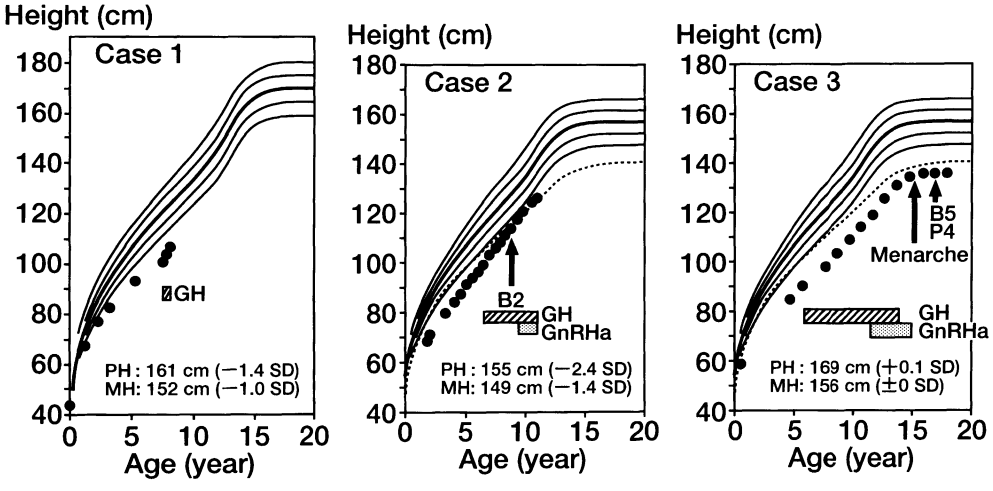

Fig. 1. Growth charts of cases 1–3 plotted on the longitudinal growth curves for Japanese children (mean, ±1 SD, and ±2 SD) [9]. Dashed lines in cases 2 and 3 represent the mean growth curve in Turner syndrome patients with spontaneous genital bleeding [10]. PH: paternal height; MH: maternal height; GH: growth hormone; GnRHa: GnRH analog; B2: breast at Tanner stage 2; B5: breast at Tanner stage 5; and P4: pubic hair at Tanner stage 4.

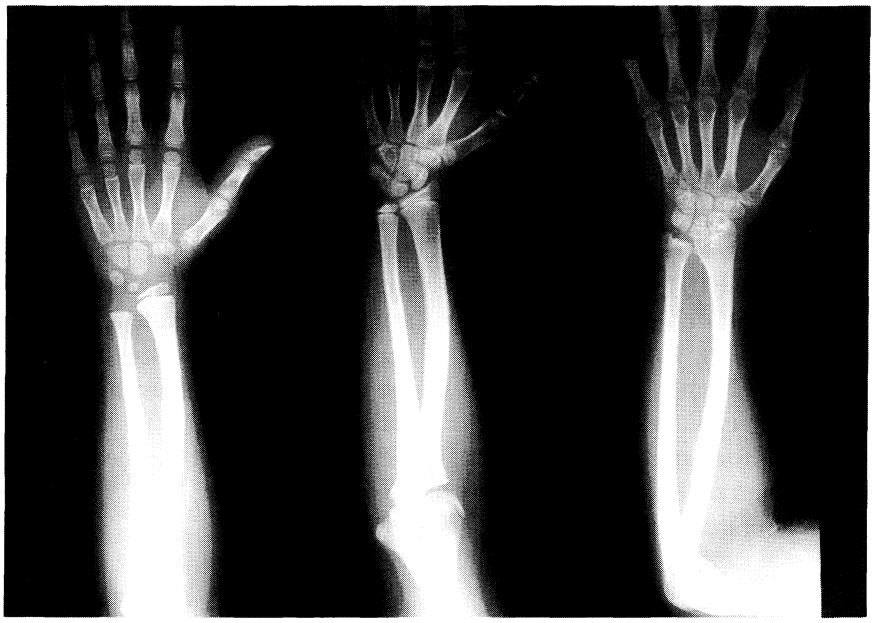

Fig. 2. Radiographs of the hands and forearms in case 1 at 7 years of age (left), case 2 at 10 years of age (middle), and case 3 at 17 years of age (right).

and MIC2 on the PAR1 as previously reported [4], demonstrating terminal deletion of the Y chromosomal PAR1 involving SHOX, with the breakpoint between DXYS59 and MIC2.

At 7 2/12 years of age, GH therapy was started at a dose of 0.5 IU (0.17 mg)/kg/week. After 12 months of treatment, his height increased from 101.0 cm (−3.9 SD) to 111.7 cm (−2.8 SD), with an increase in height velocity from 3.8 cm/year (−2.5 SD) to 10.7 cm/year (+6.4 SD) [9] (Fig. 1). BA progressed chronologically during the GH therapy.

FISH analysis was also performed for the parents and the 6 5/12-year-old sister, showing the same deletion in the Y chromosome of the father and in one of the two X chromosomes of the sister. The father was 161 cm in height (−1.7 SD) and had no definitive skeletal lesions. The sister was 106.0 cm tall (−2.0 SD) and exhibited right borderline short 4th metacarpal and bilateral mild radial curvature. The mother had two copies of SHOX and measured 152 cm (−1.2 SD).

Case 2

This girl was born at 36 weeks of gestation with a weight of 1.55 kg (−2.5 SD). She received a surgical operation for duodenal atresia at 2 days of age. At 6 6/12 years of age, she presented with short stature. The height was 97.5 cm (−3.3 SD) and the weight 14.2 kg (−2.1 SD) (Fig. 1). Psychomotor development was normal. BA was determined as 3.3 years, and hand radiographs showed no skeletal abnormalities. The peak serum GH level was 6.3, 7.2, 12.2, and 13.0 ng/ml after administration of GHRH, l-dopa, arginine, and insulin, respectively. Serum IGF-I was 125 ng/ml (60–370 ng/ml). Other laboratory and endocrine studies were normal. Her karyotype was 46,XX in 20 lymphocytes analyzed. She was enrolled in our large series of SHOX mutational analysis for idiopathic short stature. FISH analysis confirmed two copies of SHOX, and sequence analysis for leukocyte genomic DNA by the previously described method [1] revealed a heterozygous SHOX nonsense mutation (C674T) identical to that of the sibs described by Binder *et al.* [7]. The mother had no SHOX mutation, and the father refused SHOX analysis.

At 6 8/12 years of age, she was placed on GH therapy at a dose of 0.5 IU (0.17 mg)/kg/week. Her growth velocity was 4.0 cm/year (−3.0 SD) before treatment, 6.6 cm/year (+1.4 SD) in the first year, and 6.0 cm/year (+0.9 SD) in the second year of

treatment [9] (Fig. 1). She showed breast development of Tanner stage 2 at 9 3/12 years of age. At that time, basal serum estradiol (E_2) was 55 pg/ml (<10–60 pg/ml), and a GnRH test showed pubertal stage appropriate responses of serum LH (1.3→15 mIU/ml) and FSH (4.9→27 mIU/ml). BA was 9 9/12 years. GnRHa therapy was started from 9 8/12 years of age (60 μg/kg, every 4 weeks), resulting in suppression of basal serum LH (<0.5 mIU/ml) and FSH (<3 mIU/ml). At 10 10/12 years of age, her height was 126.4 cm (−2.4 SD), BA 10 6/12 years, and breast development at Tanner stage 2. There was no short metacarpal or cubitus valgus. Radiographs of the hands and forearms indicated mild shortening and borderline curvature of bilateral radii without definitive Madelung deformity (Fig. 2).

Case 3

This girl was seen by a medical practitioner at 6 years of age because of short stature (94.0 cm, −4.0 SD). Endocrine studies were informed to be normal (precise data not available), and chromosome analysis indicated a non-mosaic 46,X,der(X) karyotype in 100 lymphocytes analyzed. She was treated with GH at a dose of 0.5 IU (0.17 mg)/kg/week, as a clinical trial for sex chromosome abnormalities, from 6 to 14 years of age, and also with GnRHa (60 μg/kg, every 4 weeks) from 12 to 15 years of age. Her height remained around mean −4 SD throughout her growth period, with no discernible alteration of height velocity (Fig. 1). Menarche occurred shortly after the cessation of GnRHa therapy, and was followed by irregular menses for 1.5 years and regular menses thereafter.

She was referred to us for the examination of the X chromosomal abnormality at 17 years of age. Physical examination showed mesomelic short stature (137.4 cm, −4.1 SD), bilateral cubitus valgus, and age-appropriate pubertal development (breast, Tanner stage 5; pubic hair, Tanner stage 4). There were no other Turner stigmata or non-specific minor anomalies. Psychomotor development was normal. BA was 17 years. Basal serum LH was 3.5 mIU/ml (0.3–17.3 mIU/ml), FSH 2.8 mIU/ml (1.4–10.7 mIU/ml), and E_2 68.0 pg/ml (11–160 pg/ml). Radiographs of the hands and forearms showed bilateral decreased carpal angle, angulation of the distal radius, and shortening and curvature of radius indicative of Madelung deformity (Fig. 2). FISH analysis was performed, as previously described [4], with probes for Xp/Yp telomere region and SHOX on the PAR1, KAL1 and DXS85 on the X-differential region at Xp22.3, whole X painting, 2p telomere region, and whole chromosome 2 painting, showing a 46,X,der(X)t(X;2)(p22.3;p21) karyotype accompanied by loss of SHOX from the der(X) chromosome, with the Xp breakpoint distal to DXS85 at a position roughly 12 Mb from the Xp telomere [12]. R-banding analysis demonstrated selective X-inactivation of the der(X) chromosome and spreading of the X-inactivation into the entire translocated 2p region, indicating that the translocated 2p region was transcriptionally silent.

Discussion

SHOX haploinsufficiency was identified in cases 1–3 with short stature and Turner skeletal features of variable degrees. Since hemizygosity of the PAR1 other than SHOX has no clinical effects in both sexes, and that of the X-differential region distal to DXS85 has no clinical effects in females, especially in those with selective X-inactivation [13], the results provide further support for the notion that SHOX is relevant to both short stature and Turner skeletal features. Furthermore, the present study indicates that Madelung deformity can appear even in a prepubertal boy. This finding, as well as the development of Madelung deformity in exceptional females with 45,X Turner syndrome [14], implies the relevance of a hitherto unknown modifying factor(s) to the development of skeletal lesions in SHOX haploinsufficiency.

Cases 1–3 were treated with GH. In this context, it would be useful to compare the GH effects in cases 1–3 with those in similarly treated (0.5 IU or 0.17 mg/kg/week) Japanese patients with non-endocrine short stature (NESS) or Turner syndrome. The height velocity has been reported to be 4.4±0.8 [SD] cm/year before treatment, 7.2±1.3 cm/year in the first year, and 6.3±1.2 cm/year in the second year of treatment in NESS [15], and 4.0±1.1 cm/year before treatment, 5.9±1.1 cm/year in the first year, and 4.6±1.0 cm/year in the second year of treatment in Turner syndrome [16]. These data suggest that the 1-year GH therapy in case 1 has caused a much larger effect than that in patients with

NESS or Turner syndrome, the 2-year GH therapy in case 2 before pubertal development has resulted in an intermediate effect between patients with NESS and those with Turner syndrome, and the GH therapy in case 3 appears to have no discernible short-term or long-term effect. For case 3 with long term data, two matters may be noteworthy. First, although it has been reported that patients with obvious skeletal features such as Madelung deformity exhibit downward growth shift with puberty [4], such downward growth shift was absent in case 3 with mild but definite skeletal lesion. This may suggest a beneficial effect of GH therapy in case 3, especially in the combination with GnRHa therapy. Second, although Turner patients show gradual downward growth shift [10] because of growth disadvantage caused by chromosome imbalance (quantitative alteration of euchromatic or non-inactivated region) [17], such downward growth shift was also absent in case 3. This would be compatible with the lack of gross chromosome imbalance in case 3; the 2p segment attached onto Xp22.3 has been heterochromatized because of the spreading of X-inactivation, so that it should have no deleterious effect on development [17]. Despite such complex issues, the overall data in this study suggests that the effect of GH therapy is not necessarily promising, as in most disorders with growth deficiency including Turner syndrome.

Cases 2 and 3 received GnRHa therapy, based on the idea that GH and GnRHa combination therapy could increase the final height [18]. In this regard, skeletal lesions may be milder in cases 2 and 3 than in individuals with classic Madelung deformity [19]. It is uncertain, however, whether the GnRHa therapy has served to mitigate the skeletal lesions, because of considerable variation in the skeletal lesions among affected individuals [1–5] and insufficient data to assess the effects of GnRHa therapy in cases 2 and 3. By contrast, it is probable that cases 2 and 3 have more severe skeletal lesions than typical Turner females [20]. This may be due to the GnRHa therapy being started after pubertal entry, because such a therapy cannot prevent skeletal tissues from being exposed to gonadal estrogens in the early puberty. Considering that Madelung deformity is usually absent in Turner females who have complete gonadal dysgenesis and receive low dose estrogen therapy from a relatively late age [21], gonadal suppression in the early puberty may be important to mitigate the skeletal lesions and resultant growth failure.

For GH and GnRHa therapy in SHOX haploinsufficiency, several critical matters remain to be clarified: (1) although short term GH effect appeared variable in cases 1–3, there has been no clinical indication to discriminate between good (case 1) and poor (case 3) responders; (2) it is unknown whether the good responders to short term GH therapy could have an increased final height; (3) GH therapy may facilitate the development of skeletal anomalies by accelerating distorted skeletal growth resulting from unbalanced premature fusion [22] or by stimulating gonadal development and resultant estrogen production [23]; (4) it is unknown whether GnRHa therapy can indeed mitigate the development of severe skeletal lesions; and [5] the adequate dosage and the timing to start and stop the therapy have not been clarified, especially for GnRHa therapy. At present, therefore, it may be reasonable to attempt short term (e.g. 1-year) GH therapy and continue the therapy in good responders (e.g. patients with sufficient statural response as compared with that in patients with NESS or Turner syndrome), with careful observation for height velocity and skeletal features, and to combine GnRHa treatment at a sufficient dose from early puberty, especially in early maturing girls or in individuals with early signs of Maldelung deformity.

In summary, although GH and GnRHa therapy may be beneficial in SHOX haploinsufficiency, the clinical data are obviously limited. Further studies are necessary to evaluate the effects of GH and GnRHa therapy.

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