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Statural Growth in 31 Japanese Patients with *SHOX* Haploinsufficiency: Support for a Disadvantageous Effect of Gonadal Estrogens

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Abstract. Although gonadal estrogens are known to facilitate the development of skeletal lesion in *SHOX* haploinsufficiency, controversy exists as to whether gonadal estrogens are disadvantageous to pubertal growth. To clarify this matter, we analyzed growth pattern in 31 Japanese patients with a normal karyotype and molecularly confirmed *SHOX* haploinsufficiency. The mean height SD score at the diagnosis of SHOX haploinsufficiency was similar between patients identified in childhood and those identified in adulthood (–2.7 ± 0.8 [n = 15] vs. –2.4 ± 0.7 [n = 16], $P = 0.36$), and was significantly lower in patients identified by the studies for short stature than in those ascertained by the familial studies of the probands both in childhood (–3.0 ± 0.6 [n = 11] vs. –1.8 ± 0.5 [n = 4], $P = 0.0051$) and in adulthood (–3.0 ± 0.9 [n = 5] vs. –2.2 ± 0.5 [n = 11], $P = 0.040$). Analysis of longitudinal paired growth data obtained in seven females showed a significantly different mean height SD score between childhood and adulthood (–2.3 ± 0.5 vs. –2.9 ± 0.8, $P = 0.0060$). The results imply that gonadal estrogens have a deleterious effect on pubertal growth in *SHOX* haploinsufficiency, and that the growth disadvantage is recognizable by longitudinal rather than cross-sectional growth studies.

Key words: SHOX haploinsufficiency, Pubertal growth, Gonadal estrogen, Longitudinal analysis
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SHOX (short stature homeobox containing) gene on the short arm pseudoautosomal region of the human sex chromosomes is relevant to the development of growth failure and skeletal features in Turner syndrome. Clinical studies in patients with *SHOX* mutations or microdeletions involving *SHOX* as the sole disease gene have indicated that *SHOX* haploinsufficiency causes not only short stature but also Turner skeletal features such as short metacarpals, cubitus valgus, and Madelung deformity characteristic of Léri-Weill dyschondrosteosis (LWD) [1–8]. Since skeletal features are more severe in females than in males and become obvious with puberty, 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 severe skeletal lesions such as Madelung deformity in a female-dominant and pubertal tempo-dependent fashion [2, 4, 5].

It remains to be clarified, however, whether gonadal estrogens are disadvantageous to pubertal growth in

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patients with *SHOX* haploinsufficiency. Kosho *et al*. [2] and Fukami *et al*. [6] have reported that female patients usually grow along the –2 SD growth curve in childhood and exhibit further downward growth shift with puberty, while male patients usually grow along by the –2 SD growth curve throughout the growth period. By contrast, Ross *et al*. [5] have documented that statural growth is irrelevant to age, sex, or pubertal status. Here, we report prepubertal and postpubertal statural growth in individuals with *SHOX* haploinsufficiency, and discuss the effects of gonadal estrogens on statural growth.

Materials and Methods

Patients

This study consisted of 31 Japanese patients with molecularly confirmed *SHOX* haploinsufficiency, including 15 previously reported patients [2, 6–8]. Twenty-one patients were familial cases from seven pedigrees, in which six patients with *SHOX* haploinsufficiency (a prepubertal boy, two prepubertal girls, and three postmenarchial females) and a patient with *SHOX* nullizygosity (an infantile boy) were identified as the probands because of short stature (<–2.0 SD), and 15 patients (four prepubertal girls, five adult males, and six adult females) were ascertained through familial studies for individuals with low-normal to short stature and/or mesomelic appearance. The remaining 10 patients (eight prepubertal girls and two postmenarchial females) were sporadic cases identified because of short stature (<–2.0 SD). Thus, the 31 patients were divided into two groups in terms of the ascertainment of *SHOX* haploinsufficiency: (1) group 1, 16 patients (the six probands of familial cases and the 10 sporadic cases) identified by the studies for short stature; and (2) group 2, 15 patients ascertained through familial studies.

All the patients had a normal karyotype and were free from co-incidental disorders for short stature such as endocrine diseases. *SHOX* haploinsufficiency was caused by R195X in two sporadic cases, R168W in three cases from one family, and pseudoautosomal submicroscopic deletions involving *SHOX* in the remaining 26 patients. Molecular studies of *SHOX* haploinsufficiency were performed under the approval of the Institutional Review Board Committee of National Research Institute for Child Health and Development. Menarchial age was 12.1 ± 0.7 years in the 11 adult females (menarchial age in normal Japanese females: 12.25 ± 1.25 years) [9].

Growth assessment

Growth data were compared between childhood with no signs of pubertal development (5–8 years of age in both sexes) and adulthood with full pubertal development (>20 years of age in both sexes or postmenarchial late teens in females). The heights were evaluated by the longitudinal growth standards for the Japanese [10]. In patients receiving growth hormone and/or gonadotropin releasing hormone analog treatment, pretherapeutic clinical data only were analyzed. To allow for comparison between different ages and different sexes, the statural data were expressed as SD scores (SDSs).

The statistical significance of the mean was examined by the two-tailed Student's t-test for two unpaired groups, after examining the normality by the χ^2 test and comparing the variances by the F-test, and by the two-tailed paired t-test for two paired groups. $P<0.05$ was considered significant.

Results

The height SDSs of the 31 patients at the diagnosis of *SHOX* haploinsufficiency are shown in Fig. 1. The mean height SDS was similar between patients identified in childhood and those identified in adulthood (–2.7 ± 0.8 [n = 15] vs. –2.4 ± 0.7 [n = 16], $P = 0.36$), and between males and females of group 2 identified in adulthood (–2.1 ± 0.3 [n = 5] vs. –2.3 ± 0.6 [n = 6], $P = 0.38$). However, it was significantly lower in group 1 than in group 2 both in childhood (–3.0 ± 0.6 [n =11] vs. –1.8 ± 0.5 [n = 4], $P = 0.0051$) and in adulthood (–3.0 ± 0.9 [n = 5] vs. –2.2 ± 0.5 [n = 11], $P = 0.040$).

The longitudinal height SDSs obtained in seven patients are shown in Fig. 2. Growth data were taken prospectively in two girls and retrospectively in five adult females. Statural growth invariably deteriorated in all the seven patients, with the significantly different mean height SDS between childhood and adulthood (–2.3 ± 0.5 vs. –2.9 ± 0.8, $P = 0.0060$).

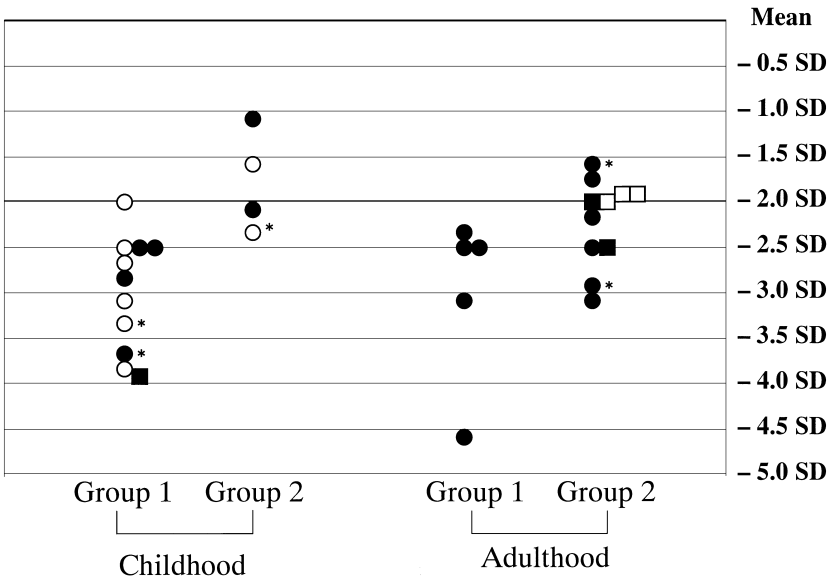

Fig. 1. Height SD scores of 31 patients at the time of the diagnosis of *SHOX* haploinsufficiency. Fifteen patients have been identified in childhood, and 16 patients have been recognized in adulthood. Group 1 consists of six probands of familial cases and 10 sporadic cases identified by the studies for short stature, and group 2 is comprised of 15 patients ascertained through familial studies. Open and black circles indicate female patients with and without radiological findings of Madelung deformity, respectively. Open and black squares depict male patients with and without radiological findings of Madelung deformity, respectively. Five patients marked with asterisks have intragenic *SHOX* mutations, and the remaining patients have pseudoautosomal microdeletions involving *SHOX*.

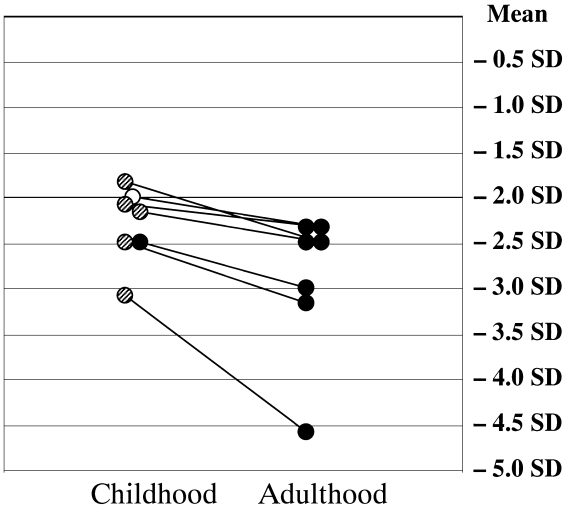

Fig. 2. Changes of height SDSs between childhood and adulthood in seven female patients. Growth data have been obtained prospectively in two females and retrospectively in five females. In childhood, radiological findings of Madelung deformity were detected in a female (a closed circle) and undetected in another female (an open circle); striped circles represent five females who were first identified in adulthood, so that retrospective growth data were available but radiological findings were unexamined in childhood. In adulthood, radiological findings of Madelung deformity were exhibited by all seven females.

Discussion

Growth analysis of the paired data in the seven patients has shown downward growth shift of ~0.6 SD between childhood and adulthood. This provides further support for the notion that gonadal estrogens exert a maturing effect on skeletal tissues vulnerable to premature growth plate fusion because of *SHOX* haploinsufficiency, worsening skeletal lesion and resultant growth failure with puberty [2, 4]. Although auxological data such as arm span and leg length were not available in most of the 31 patients, the previous longitudinal study in a female with *SHOX* haploinsufficiency [6], in conjunction with *SHOX* expression in the long bones of the forearms and shanks as well as in the first and second pharyngeal arches of the human embryos [3], implies that growth deficiency during puberty is primarily due to deterioration of mesomelia.

By contrast, statural analysis at the diagnosis of *SHOX* haploinsufficiency in the 31 patients has failed to reveal growth deficiency during puberty. In this regard, it is noteworthy that growth failure was more severe in group 1 than in group 2 in both childhood and adulthood. This would imply the presence of an ascertainment bias that patients with relatively severe

growth failure are frequently identified in childhood by the studies for short stature whereas those with relatively mild short stature often remain undetected in childhood and are recognized in adulthood after downward growth shift during puberty by the familial studies. This notion would explain why the height SDSs were similar between childhood and adulthood in the 31 patients. Furthermore, this notion may also explain why the height SDSs were irrelevant to age, sex, or pubertal status in the study of Ross *et al*. [5], because they examined the height SDSs at the time of diagnosis of *SHOX* haploinsufficiency.

In addition, two matters are notable in the present study. First, the adult height SDSs were similar between males and females in group 2. Since females have a larger amount of gonadal estrogens and enter puberty earlier than males, this may argue against the deleterious effect of gonadal estrogens on the pubertal height gain. However, since familial studies were preferentially carried out for individuals with low-normal to short stature and/or mesomelic appearance predominantly manifested by female patients, it is possible that males with apparently normal phenotype remained unrecognized. Second, most patients in group 1 were females, even in childhood. Although the reason for such a distorted sex ratio is unknown, one possibility might be that serum estrogen levels are higher in girls than in boys from childhood [11].

In summary, the present study suggests that gonadal estrogens are disadvantageous to pubertal growth in *SHOX* haploinsufficiency, and that the growth disadvantage is recognizable by longitudinal rather than cross-sectional growth studies. Further longitudinal growth studies in a large number of non-biased individuals will permit a better characterization of pubertal growth in *SHOX* haploinsufficiency.

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