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Skeletal Features and Growth Patterns in 14 Patients with Haploinsufficiency of SHOX: Implications for the Development of Turner Syndrome

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ABSTRACT

We report on clinical features in 14 Japanese patients (4 males and 10 females) with partial monosomy of the short arm pseudoautosomal region involving SHOX (n = 11) or total monosomy of the pseudoautosomal region with no involvement of disease genes on the sex-differential regions (n = 3). Skeletal assessment showed that three patients had no discernible skeletal abnormalities, one patient exhibited short 4th metacarpals and borderline cubitus valgus, and the remaining 10 patients had Madelung deformity and/or mesomelia characteristic of Léri-Weill dyschondrosteosis (LWD), together with short 4th metacarpals and/or cubitus valgus. Skeletal lesions were more severe in females and became obvious with age. Growth evaluation revealed that patients without LWD grew along by the −2 SD growth curve before puberty and showed a normal or exaggerated pubertal growth spurt, whereas those with LWD grew along by the standard growth curves before puberty but exhibited an attenuated pubertal growth spurt and resultant short stature. Maturational assessment indicated a tendency of relatively early maturation in patients with LWD. There was no correlation between the clinical phenotype and the deletion size.

These findings suggest that haploinsufficiency of SHOX causes not only short stature but also Turner skeletal anomalies (such as short 4th metacarpals, cubitus valgus, and LWD) and that growth pattern is primarily dependent on the presence or absence of LWD. Because skeletal lesions have occurred in a female-dominant and age-influenced fashion, it is inferred that estrogens exert a maturational effect on skeletal tissues that are susceptible to premature fusion of growth plates because of haploinsufficiency of SHOX, facilitating the development of skeletal lesions. (*J Clin Endocrinol Metab* **84:** 4613–4621, 1999)

TURNER SYNDROME is a well-defined sex chromosomal disorder associated with short stature and various skeletal anomalies, together with nonskeletal stigmata. The incidence is nearly 100% for short stature and 35–60% for representative skeletal anomalies such as short metacarpals, cubitus valgus, high-arched palate, micrognathia, and short neck (1). In addition, Léri-Weill dyschondrosteosis (LWD) characterized by Madelung deformity and mesomelia occasionally occurs in Turner syndrome (1).

A gene for short stature has been postulated to be on the short arm pseudoautosomal region (PAR1) of the sex chromosomes (2). Recently, Rao *et al.* (3) cloned a novel gene from the distal part of the PAR1, and named it SHOX for short stature homeobox-containing gene. This gene has also been identified by Ellison *et al.* (4) and termed PHOG for pseudoautosomal homeobox-containing osteogenic gene. SHOX is most strongly expressed in bone marrow fibroblasts, implying that SHOX plays a positive role in bone growth and development (3, 4). In addition, SHOX is expressed on both the inactive X chromosome as well as an active X and Y chromosome, suggesting that SHOX escapes X-inactivation and exerts a dosage effect in patients with sex chromosome aberrations (3). Furthermore, Rao *et al.* (3) have found a heterozygous nonsense mutation of SHOX cosegregating with short stature phenotype in a German family, thereby providing compelling evidence for SHOX being the growth gene.

Subsequently, haploinsufficiency of SHOX has also been shown to cause LWD. Point mutations of SHOX were identified in two families with LWD, and microdeletions involving SHOX were found in 12 families with LWD (5, 6). The high prevalence of microdeletions would be consistent with repetitive sequences such as subtelomeric interspersed repeats being abundantly present around SHOX (7), because unequal

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crossing over between homologous chromosomes or an intrachromosomal recombination is prone to occur in such a region.

However, several matters remain to be determined for the skeletal features in Turner syndrome, including the cause of skeletal features other than short stature and LWD, the underlying factor(s) for the phenotypic variation in SHOX haploinsufficiency, and the growth patterns in SHOX haploinsufficiency. Here, we report on clinical features in 14 patients with partial or total monosomy of the PAR1 involving SHOX and discuss on the unresolved issues on the skeletal features in Turner syndrome.

Materials and Methods

Patients

Fourteen Japanese patients were collected from a large series of patients with sex chromosome aberrations and/or those with LWD. The selection criteria used were: 1) records of clinical assessment; 2) lack of apparently coincidental disorders such as endocrine diseases; 3) no therapeutic intervention that may affect skeletal growth or development; 4) lack of demonstrable mosaicism; 5) absence of autosomal euchromatic abnormalities; 6) partial monosomy of the PAR1 or total monosomy of the PAR1 with no involvement of disease genes on the sex-differential regions; and 7) demonstration of SHOX haploinsufficiency (the details of clinical assessment and molecular studies are reported here). Sex, age, and karyotype of each patient are shown in Table 1. Cases 1–7, 10, 12, and 14 were identified because of short stature, and the remaining cases were ascertained through familial study of the probands. Cytogenetic and/or molecular studies of the family members showed that the pseudoautosomal deletions were sporadic in cases 1–6 and familial in cases 7–14 (Table 1). The father of cases 13 and 14 (family 4) was deceased and, allegedly, he had borderline short stature. Cases 3, 6, and 7 have been reported previously (8–10). All cases were free from nonskeletal Turner stigmata such as webbed neck, puffy hands and feet, aortic coarctation, and horseshoe kidney.

Clinical assessment

Skeletal lesion, growth pattern, and sexual maturation were examined. For the skeletal evaluation, short 4th metacarpals, cubitus valgus, Madelung deformity, mesomelia, genu valgum, high-arched palate, micrognathia, and short neck were assessed clinically. In addition, short 4th metacarpal was radiologically evaluated as positive, borderline, or negative when a tip of the 4th metacarpal is below, on, or above a straight line drawn between tips of the 3rd and 5th metacarpals, respectively (1). Madelung deformity and mesomelia were also radiologically assessed for several indications such as lucency at the ulnar border of the distal radius, decreased carpal angle formed by the tangents of the scaphoid-lunate and triquetrum-lunate peripheries, angulation of the distal radius and ulna, and shortening and curvature of radius (11).

For the growth evaluation, the birth size was assessed by the gestational age-matched Japanese standards and the height was evaluated by the longitudinal growth standards for the Japanese (12). Predicted adult height (PAH) was calculated by the method of Ito and Yokoya (13) (a modified Bayley-Pinneau's method for the Japanese) for patients who have not attained the final height [PAH is not obtained in children with bone age (BA) less than 5 yr]. Target height (TH) and target range (TR) were obtained from the equations of Ogata *et al.* (14) (a modified Tanner's equation for the Japanese with a positive height secular trend) for patients with *de novo* deletions (TH/TR is not obtained in patients with familial SHOX deletions because it predicts the adult height/range of a child born to normal parents).

For the maturational evaluation, pubertal stage (genitalia in males, breast in females, and pubic hair in both sexes) was assessed by the classification of Tanner (15) and BA was evaluated by the skeletal atlas of the hand and wrist for the Japanese (16). Menarchial age was also obtained in females. The pubertal stage data were compared with those of normal Japanese (17).

Fluorescence in situ *hybridization (FISH) analysis*

Probes for SHOX (a ~18-kb cosmid covering a region from intron 1 to intron 5A), DXYS59 (18), and the Xp/Yp telomeric region (19) were hybridized to lymphocyte metaphase spreads of cases 1–14, the parents of cases 4 and 5, the mothers of families 2–4, and the brother of family 2. The physical positions of examined loci/region on the PAR1 are shown in Fig. 1. In addition, a probe for KAL (Kallmann syndrome) located in the X-differential region at Xp22.3 was hybridized to metaphase spreads of cases 4, 7, and 8. For an internal signal control, a probe for the Xq/Yq telomeric region (19) was concomitantly hybridized to metaphase spreads. The probe for the Xq/Yq telomeric region was labeled with biotin and was detected by avidin conjugated to fluorescein isothiocyanate, and the remaining probes were labeled with digoxigenin and were detected by rhodamine antidigoxigenin.

DNA analysis

Fifteen loci on the PAR1 were determined for the copy number by PCR-based microsatellite, 4 bp insertion/deletion, and restriction fragment length polymorphism (RFLP) analyses, and by Southern blot RFLP analysis, using leukocyte genomic DNA obtained from cases 1–14, the parents of cases 1 and 3–5, the mothers of case 6 and families 2–4, and the brother of family 2. For microsatellite analysis (DXYS233, SHOX, DXS9900, DXYS228, and DXYS232) and 4-bp insertion/deletion analysis (DXYS85 and MIC2), 0.3 μg DNA was amplified by PCR with a fluorescently labeled forward primer and an unlabeled reverse primer, and the size of the PCR products was determined on an autosequencer (ABI PRISM 310; Applied Biosystems, Perkin Elmer, Norwalk, CT) using GeneScan. For PCR-RFLP analysis (DXYS15 and DXYS77), 0.5 μg DNA was amplified with unlabeled primers, and the PCR products were digested with Fnu4HI (DXYS15) or *Stu*I (DXYS77) and were loaded onto a 3% NuSieve gel (FMC BioProducts, Rockland, ME) mixed with a standard agarose gel (3:1). The primer sequences and the PCR conditions have been reported previously (5, 21) and in Genome Database. For Southern blot RFLP analysis, 10 μg DNA was digested with *Eco*RI, *Taq*I, or *Hin*dIII and was hybridized with 29C1 (DXYS14), U7A (DXYS60), P99 (DXYS87), B6 (DXYS161), 113D (DXYS15), c-DNA (CSF2RA), 601 (DXYS17), and 19B (MIC2) (18, 22). The physical positions of examined loci on the PAR1, except for DXS9900, DXYS228, and DXYS232, are shown in Fig. 1.

Furthermore, PCR analysis was carried out for PABX (pseudoautosomal boundary on the X chromosome) and ARSE (X-linked recessive chondrodysplasia punctata) in cases 7 and 8 (family 1), and microsatellite analysis was performed for DXS996 in case 4 and her parents. The locus order is: Xpter–(PAR1)–PABX–ARSE–DXS996–(KAL)–cen. The primer sequences and the PCR condition were as reported previously (23, 24) and in Genome Database.

Results

Clinical assessment

Skeletal lesions are summarized in Table 1, and representative radiographs are shown in Fig. 2. Cases 1, 2, and 7 were free from skeletal abnormalities, case 3 had short 4th metacarpals and borderline cubitus valgus but was free from Madelung deformity or mesomelia, and the remaining cases had not only short 4th metacarpals and/or cubitus valgus of variable degree but also Madelung deformity and/or mesomelia of variable extent. Skeletal lesions tended to be more severe in females than in males and became distinct with age. For example, the sex difference was displayed in families 1–3, and the age difference was manifested in family 4. Other skeletal features were absent, except for high-arched palate and genu valgum in case 12.

Growth data are summarized in Table 1, and the growth charts of cases 1–7, 10, and 12–14 are shown in Fig. 3, together with PAH and TH/TR. At birth, the length and weight were below the mean in most cases; the length SD score ranged

TABLE 1. Summary of patients examined in the present study

Patients					Skeletal lesions				Growth data[a]					
Case	Sex	Age (yr:month)	Karotype (analyzed cell number)	Remark	Short 4th metacarpals	Cubitus vulgas	Madelung deformity	Mesomelia	BL (cm)	BW (kg)	GA (wk)	AH[b] (cm)	TH ± range (cm)	PAH (cm)
1	M	9:3	46,X,der(Y)(qter→q11.2::p11.3→qter)[100]	*De novo*	No	No	No	No	48.3 (−1.4)	3.3 (+0.1)	40	120.5 (−2.0)	165 ± 9 (−0.8 ± 1.6)	155.5 (−2.4)
2	F	2:0	46,X,Xps[50]	*De novo*	No	No	No	No	46.5 (−1.8)	2.6 (−1.5)	40	78.4 (−2.0)	153 ± 8 (−1.0 ± 1.6)	...
3	F	19:2	46,X,der(X)(qter→q26::p22.3→q26:)[50]	*De novo*	Yes	Borderline	No	No	47.9 (−1.0)	3.2 (−0.1)	41	153.5 (−0.9)	163 ± 8 (+1.1 ± 1.6)	...
4	F	3:3	46,X,del(X)(p22.32)[100]	*De novo*	No	Yes[c]	Yes[c]	Yes[d]	46.0 (−2.0)	3.0 (−0.5)	40	83.8 (−2.6)	160.5 ± 8 (+0.5 ± 1.6)	...
5	F	10:8	46,XX[50]	*De novo*	Yes	Yes[e]	Yes[d]	Yes[c]	50.0 (+0.3)	3.6 (+1.1)	40	124.5 (−2.5)	162 ± 8 (+0.9 ± 1.6)	144 (−2.8)
6	F	14:8	46,X,der(X)(qter→p22.3::p22.3→p21.3:)[263]	*De novo*	Yes	Borderline	Yes[c]	No	48.0 (−1.4)	2.2 (−2.6)	40	139.6 (−3.3)	155.5 ± 8 (−0.5 ± 1.6)	141 (−3.4)
7	M	18:0	46,Y,der(X)t(X;Y)(p22.3;q11)[50]	Familial-1	No	No	No	No	49.2 (−0.7)	3.0 (−0.8)	40	158.7 (−2.1)	...	...
8	F	42:5	46,X,der(X)t(X;Y)(p22.3;q11)[50]	Familial-1	Borderline	Yes	Yes[c]	Yes[c]	...	...	...	142.0 (−3.2)	...	...
9	M	43:5	46,XY[50]	Familial-2	Borderline	No	Borderline	Yes[c]	...	...	...	160.3 (−1.9)	...	...
10	F	12:10	46,XX[50]	Familial-2	Yes	Yes[e]	Yes[e]	Yes[e]	46.5 (−1.8)	3.0 (−0.5)	40	135.4 (−3.1)	...	137 (−4.1)
11	M	43:2	46,XY[50]	Familial-3	Yes	Yes	Yes[c]	Yes[d]	...	...	...	159.2 (−2.0)	...	...
12	F	19:2	46,XX[100]	Familial-3	Yes	Yes[e]	Yes[e]	Yes[e]	46.0 (−2.0)	3.0 (−0.5)	40	134.8 (−4.6)	...	...
13	F	8:3	46,XX[50]	Familial-4	Borderline	Yes[c]	Yes[c]	No	49.0 (±0)	2.9 (−0.5)	39	119.8 (−1.1)	...	151 (−1.4)
14	F	11:6	46,XX[50]	Familial-4	Yes	Yes[e]	Yes[e]	Yes[e]	48.0 (−0.1)	2.8 (−0.5)	38	130.5 (−2.5)	...	140 (−3.6)

BL, birth length; BW, birth weight; GA, gestational age; AH, actual height.
Case 7 is the son of case 8 (family 1), case 9 is the father of case 10 (family 2), case 11 is the father of case 12 (family 3), and case 13 is the sister of case 14 (family 4).
[a] The values in *parentheses* represent SD scores.
[b] The age at the height measurement is shown in the third column.
[c] Mild manifestation.
[d] Moderate manifestation.
[e] Severe manifestation. Case 12 also has high-arched palate and genu valgum.

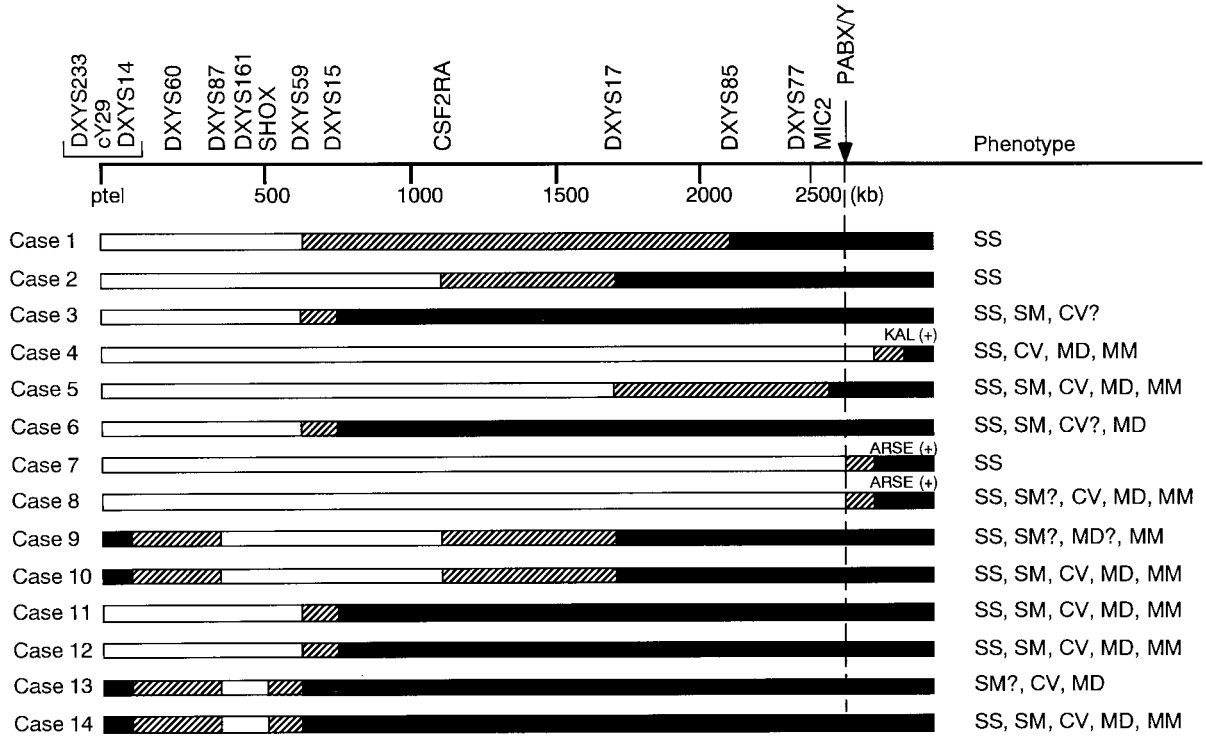

FIG. 1. Deletion maps of the short arm PAR1. The case numbers correspond to those in Table 1. The *white* and *black* areas denote the monosomic and the disomic regions, respectively, except for case 6 in whom the *black* area indicates the trisomic region. The *striped* areas depict the dosage unknown regions where the breakpoints should exist. The locus order is based on the report of Vogt *et al.* (20). The Xp/Yp telomeric region examined by FISH is indicated by the probe name detecting that region (cY29). DXS9900, DXYS228, and DXYS232 are not indicated in the figure because the precise physical location remains unknown (however, according to linkage analysis, DXYS228 and DXYS232 are assigned between MIC2 and PABX/Y) (5). The informative results have confirmed that DXS9900 is present in a single copy in cases 5, 9, and 10, and in two copies in cases 6 and 11–14, and that DXYS228 is present in two copies in cases 1 and 9–14; the results of DXYS232 were not informative in all cases. The phenotype of each case is shown on the right side (SS, short stature; SM, short 4th metacarpals; CV, cubitus vulgas; MD, Madelung deformity; MM, mesomelia).

from +0.3 to −2.0 (with the mean SD score of −1.1), and the weight SD score ranged from +1.1 to −2.6 (with the mean SD score of −0.6). Before puberty, the growth patterns were similar among patients. All the 11 cases grew along by the standard growth curves, with the heights ranging from −1.1 SD (case 13) to −3.0 SD (case 12). PAH of prepubertal patients (cases 1, 5, and 13) were around the mean −2 SD and below their TR. From puberty, the growth patterns became variable among patients. Without LWD, case 7 continued to grow along by the −2 SD growth curve, and case 3 exhibited upward growth shift; their nearly final heights were around the mean −2 SD or the lower limit of TR. With LWD, cases 6, 10, 12, and 14 exhibited a pubertal growth spurt that was, however, followed by definite downward growth shift; their PAH or nearly final height were below the mean −2 SD and TR. For the adult patients, male cases 9 and 11 had borderline short stature, and female case 8 had definite short stature.

The Tanner pubertal stages and BA in cases 1–7, 10, and 12–14 are given in Fig. 3, together with menarchial ages in cases 3, 6, 10, 12, and 14. Male patients were not remarkable for sexual maturation. Case 7 matured with an average pubertal tempo and BA progression, and cases 9 and 11 showed full sexual maturation. Female patients showed somewhat variable pubertal development, with a tendency of relatively early maturation in patients with LWD. Case 3 without LWD had menarche at the age of 13.5 yr (+1.0 SD), whereas cases 6, 8, 10, 12, and 14 with LWD experienced menarche at the age of 11.25 yr (−0.8 SD), 11.0 yr (−1.0 SD), 11.5 yr (−0.5 SD), 12.25 yr (±0 SD), and 11.4 yr (–0.7 SD), respectively (menarchial age in Japanese girls, 12.25 ± 1.25 yr). In addition, relatively rapid pubertal BA progression was suggested in cases 6 and 10.

SHOX deletions

Representative results are shown in Fig. 4. FISH analysis showed that SHOX was present in a single copy in all the 14 cases. In cases 9 and 11 with an XY sex chromosome complement, SHOX was deleted from the apparently normal Y chromosomes. In addition, SHOX was present in two copies in the parents of cases 4 and 5, the mothers of families 2–4, and the brother of family 2. Microsatellite analysis also confirmed SHOX deletions in cases 3–12.

Deletion maps

The deletion maps determined by FISH and polymorphism analyses are shown in Fig. 1. Cases 1–3, 5, 6, and 9–14 had partial monosomy of the PAR1 of variable sizes. The breakpoints resided between DXYS59 and DXYS85 in case 1; between CSF2RA and DXYS17 in case 2; between DXYS59

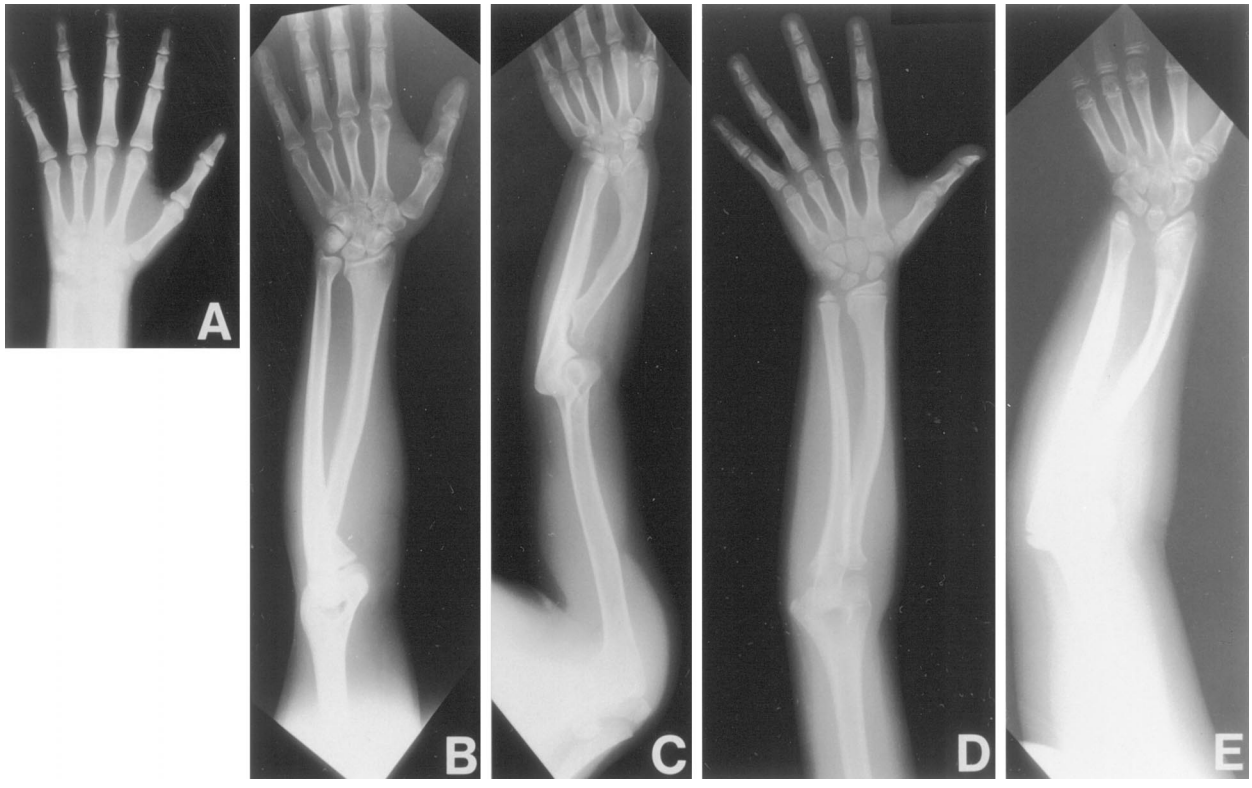

FIG. 2. Representative radiographs. A, Left hand of case 3 at 14.3 yr of age, showing short 4th metacarpal, which remained at a borderline level at 10 yr of age when she was reported previously (8). B, Left forearm of case 9 (family 2) at 43 yr of age, showing borderline short 4th metacarpal, mildly decreased carpal angle, and slightly shortened and bowed radius. C, Left forearm of case 10 (family 2, the daughter of case 9) at 12.9 yr of age, showing short 4th metacarpal, cubitus valgus, decreased carpal angle, angulation of the distal radius, and short and curved radius. D, Left forearm of case 13 (family 4) at 8.3 yr of age, exhibiting borderline short 4th metacarpal, mild cubitus valgus, and deformation of the medial half of the distal radius. E, Left forearm of case 14 (family 4, the elder sister of case 13) at 11.6 yr of age, showing short 4th metacarpal, cubitus valgus, decreased carpal angle, angulation of the distal radius, and short and curved radius.

and DXYS15 in cases 3, 6, 11, and 12; between DXYS17 and MIC2 in case 5; between the telomere region and DXYS87 and between CSF2RA and DXYS17 in cases 9 and 10; and between the telomere region and DXYS87 and between SHOX and DXYS59 in cases 13 and 14. The remaining cases (4, 7, and 8) had total monosomy of the PAR1. The breakpoint was located between DXS996 and KAL in case 4, and between PABX and ARSE in cases 7 and 8. In addition, polymorphism analysis showed that the PAR1 deletions in sporadic cases were of paternal origin in cases 3–5 and of maternal origin in case 6 (the results of case 1 were not informative for all loci examined, although the rearranged Y chromosome in case 1 should be derived from the father).

Discussion

Phenotypic spectrum

Eleven of the 14 cases had partial monosomy of the PAR1. In addition, the remaining cases (4, 7, and 8) with total monosomy of the PAR1 obviously preserved disease genes on the sex-differential region because ARSE represents the most distal disease gene in the X-differential region at Xp22.3 in males, and loss of X-differential region at Xp22.3 distal to KAL is unlikely to have clinical effects in females (25). Thus, the results allow us to analyze the clinical features in various pseudoautosomal deletions involving SHOX.

The pseudoautosomal deletions involving SHOX were associated with short stature, short 4th metacarpals, cubitus valgus, Madelung deformity, and/or mesomelia. In this regard, case 3 with short 4th metacarpals and borderline cubitus valgus had borderline short stature as compared with her TH/TR, and 10 cases with Madelung deformity and/or mesomelia had short stature (except for case 13), short 4th metacarpals, and/or cubitus valgus. It is unlikely that the phenotypic variation is due to contiguous gene deletions because there was no correlation between the phenotype and the deletion size (Fig. 1). Furthermore, intragenic SHOX mutations have been shown to result in both short stature and LWD (3, 5, 6). Thus, although an intragenic SHOX mutation has not been identified in patients with short 4th metacarpals and/or cubitus valgus, the results imply that haploinsufficiency of SHOX causes not only short stature and LWD but also limb skeletal anomalies such as short 4th metacarpals and cubitus valgus, perhaps as an intermediate feature between short stature and LWD. Consistent with this, cubitus valgus has been described in two females with terminal Xp22.3 deletions distal to KAL (26, 27).

In Turner syndrome, skeletal anomalies also appear in the faciocervical region (1). In this context, high-arched palate in case 12 may suggest that haploinsufficiency of SHOX is also involved in the development of Turner skeletal features in

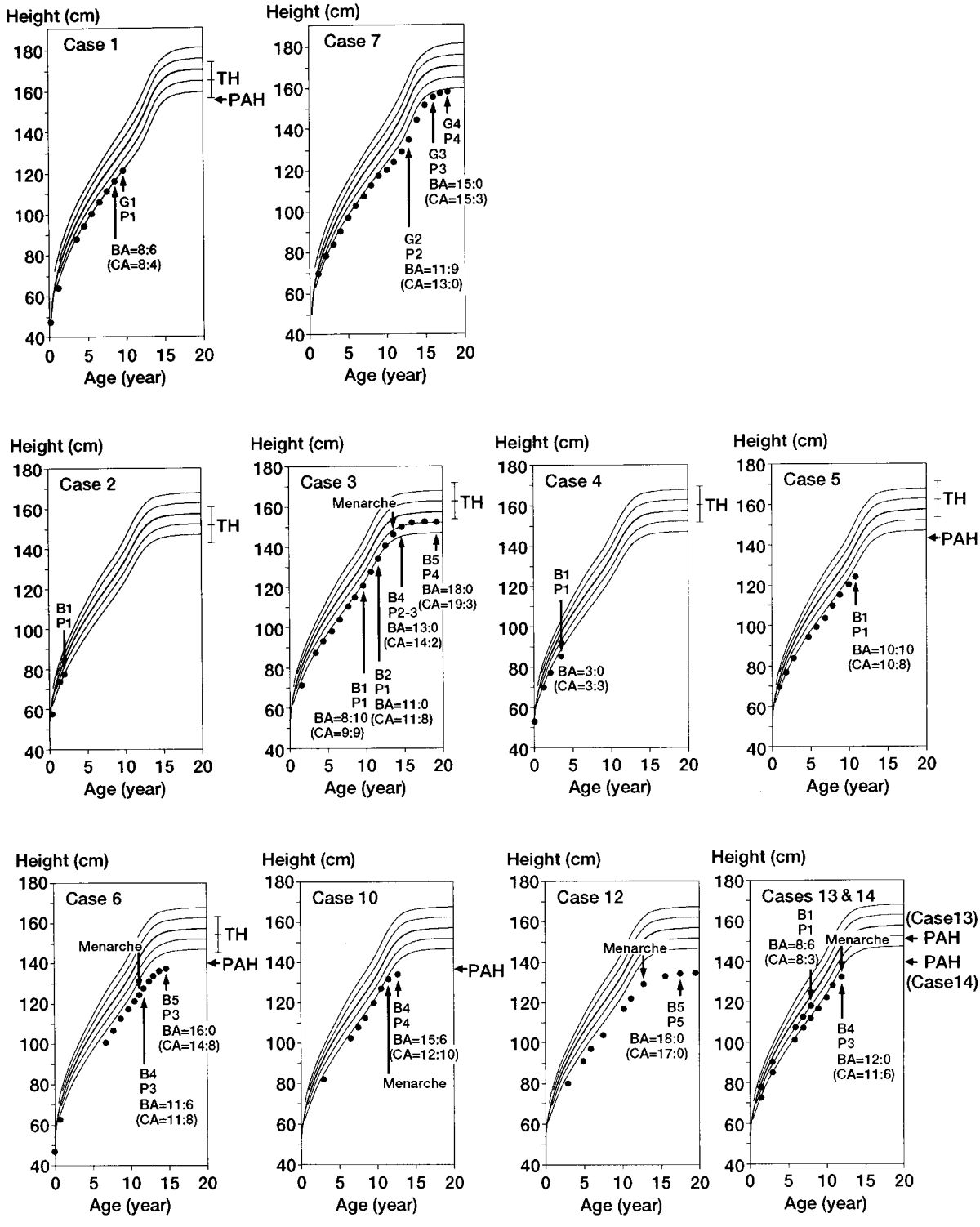

FIG. 3. Growth charts and maturational patterns of cases 1–7, 10, and 12–14. The case numbers corresponds to those in Table 1. Cases 1 and 7 are males, and the remaining cases are females. The heights of each patient (●) are plotted on the sex-matched standard growth curves (+2 SD, +1 SD; mean, −1 SD and −2 SD) for Japanese children. TH is shown in cases 1–6, together with TR (indicated by the *vertical bars*). PAH is shown in cases 1, 5, 6, 10, 13, and 14. The pubertal stage is indicated according to the classification of Tanner (G, genitalia; B, breast; P, pubic hair), together with menarchial age in cases 3, 6, 10, 12, and 14. The reference data for maturational patterns for the Japanese are as follows (mean ± SD in years): 1) boys, P2 12.5 ± 0.9, P3 14.0 ± 0.3, P4 14.9 ± 0.3, P5 not available (a considerable part of normal Japanese males do not reach to the P5 stage); G2–5, not available; and 2) girls, P2 11.7 ± 1.6, P3 not available, P4 13.9 ± 1.0, P5 not available (a considerable part of normal Japanese females do not reach to the P5 stage); B2 10.0 ± 1.4, B3 11.6 ± 1.5, B4 13.3 ± 1.5, B5 14.2 ± 1.2 (Ref. 17 and our unpublished observations). Menarchial age in Japanese girls is 12.25 ± 1.25 yr (17). BA, as assessed by the Japanese skeletal atlas (16), are given, together with chronological ages (CA), at the time of BA determination.

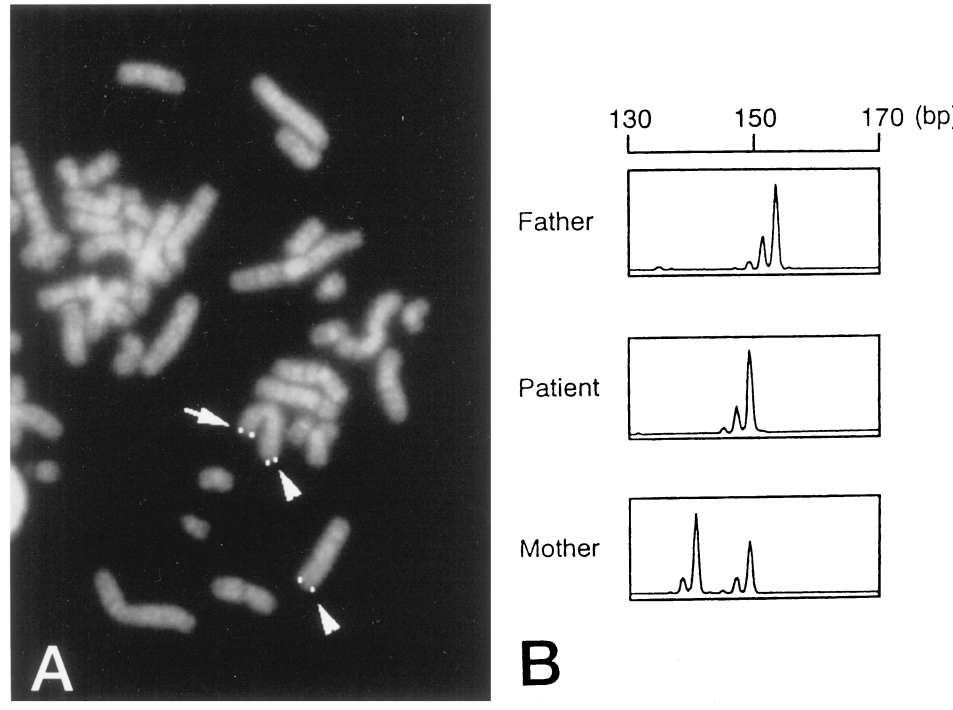

FIG. 4. SHOX analysis in case 5. A, FISH analysis. SHOX is detected in a single copy (*arrow*), whereas the Xq/Yq telomeric region are present in two copies (*arrowheads*). B, Microsatellite analysis. The paternal peak is not inherited by the patient, although one of the two maternal peaks is transmitted to the patient. Because SHOX has been confirmed to be present in two copies in both parents by FISH (data not shown), this indicates that the SHOX deletion is a *de novo* abnormality occurring in the paternally derived X chromosome.

the faciocervical region. However, high-arched palate was observed in a single patient with LWD, and other skeletal features such as micrognathia and short neck were absent in all of the 14 cases. Thus, it appears unlikely that haploinsufficiency of SHOX constitutes the major factor for Turner skeletal features in the faciocervical region. Rather, it may be that, in Turner syndrome, cystic hygroma and facial edema exert a deformational effect on the developing skeletal tissues, contributing to the development of skeletal features in the faciocervical region.

Skeletal features

Skeletal features tended to be more severe in females than in males and became more obvious after puberty. Thus, at a later age, cases 1 and 2 may have skeletal features, and cases 4, 5, and 13 may exhibit more severe skeletal features. Although this study consisted of a small number of patients and was associated with a selection bias for LWD, such female-dominant manifestation has been described for LWD (28), and age-influenced manifestation has been reported for short 4th metacarpals, cubitus valgus, and LWD (29, 30). It is inferred, therefore, that a factor(s) for sex- and age-related expression is operating in the development of skeletal lesions.

In considering the underlying factor(s) for the development of skeletal lesions, several findings are noteworthy. First, short 4th metacarpals in Turner syndrome are often associated with radiologically discernible premature fusion of the epiphyses (30, 31). Second, Madelung deformity is primarily ascribed to premature fusion of the medial half of the distal radial growth plates (32). Third, skeletal maturation, including growth plate fusion, is caused by estrogens, not by androgens, in both sexes (33, 34). Fourth, serum estrogens are higher in females than in males from infancy and increase from puberty in both sexes (35, 36). Thus, it is likely that estrogens exert a maturational effect on skeletal tissues that are susceptible to premature fusion of growth plates because of haploinsufficiency of SHOX, facilitating the development of skeletal lesions in a female-dominant and age-influenced fashion. In agreement with this, maturational assessment, including menarchial age, suggests that cases 6, 8, 10, 12, and 14 with LWD tended to show early sexual maturation, which should result in early exposure to estrogens, whereas case 3 without LWD manifested relatively late sexual maturation. Furthermore, this notion would explain why the prevalence of skeletal lesions in Turner syndrome remains roughly 40% for short metacarpals and cubitus valgus and only about 7% for Madelung deformity (1), because gonadal estrogen production is usually severely compromised in Turner syndrome. One may argue against this hypothesis because Turner patients treated with estrogens rarely have Madelung deformity. However, estrogen therapy in Turner patients is usually started from late teens with a low dosage (37). This may be related to the rarity of overt skeletal lesions such as Madelung deformity in such patients.

However, a factor(s) other than estrogens would also be relevant to the development of skeletal lesions. LWD can appear in 45,X patients and in prepubertal patients (29, 38). On the other hand, LWD has not been described in several fertile females with SHOX haploinsufficiency (39). The present study also showed prepubertal development of mild LWD and variable skeletal features in sex-matched adult patients. However, such a factor(s) modifying the effect of SHOX deletions other than estrogens remains to be clarified.

Growth patterns

The birth size tended to be small, with the mean SD score being −1.1 for length and −0.6 for weight. This suggests that

growth failure in patients with SHOX haploinsufficiency may become evident *in utero*. In this regard, it is noteworthy that, in 45,X Turner syndrome, the mean SD score has been reported to be −1.01 for birth length and −1.20 for birth weight (40). Thus, although additional studies are necessary to draw a definite conclusion, it is possible that SHOX haploinsufficiency accounts for most of the birth length reduction and roughly half of the birth weight reduction in 45,X Turner syndrome.

Patients without LWD continued to grow along by the −2 SD growth curve (cases 1, 2, and 7) or showed upward growth shift with puberty (case 3), with the adult heights or PAH being around the mean −2 SD or the lower limit of TR (cases 1, 3, and 7). Consistent with the relatively mild statural effect of SHOX deletions (about 2 SD) as compared with the magnitude of normal height variation (±2 SD, *i.e.* 4 SD), the adult height of case 3 remained within the normal range, although her adult height was below her TR. This indicates that haploinsufficiency of SHOX allows normal height in patients with a high genetic height potential. Furthermore, the linear growth pattern with a normal growth rate seems to be characteristic of haploinsufficiency of SHOX because most diseases affecting growth, such as endocrine disorders, are usually associated with a reduced growth rate (41). However, the linear growth pattern is similar to that of normal variant short stature as the lower extreme of the normal height variation and, at present, there has been no indication to distinguish haploinsufficiency of SHOX from normal variant short stature.

By contrast, patients with LWD showed different growth patterns between prepubertal and pubertal periods. Before puberty, they roughly grew along by the standard growth curves (cases 4–6, 10, and 12–14) and, notably, case 13 manifested normal growth. After the onset of puberty, they showed an obvious downward growth shift and had severe short adult heights or PAH (cases 6, 10, 12, and 14). Thus, it is likely that cases 4, 5, and 13 will manifest a downward growth shift after puberty. The linear growth pattern would be explained by assuming that prepubertal growth is relatively well preserved (and can even be normal as in case 13) because of dormant gonadal function, whereas pubertal growth is compromised because of production of gonadal estrogens facilitating growth plate fusion.

The results support the notion that the growth failure and the growth pattern in 45,X Turner syndrome are inexplicable by haploinsufficiency of SHOX alone (2). In 45,X Turner syndrome, the mean adult height is about mean −3.2 SD of normal females (2, 40), and the linear growth is accompanied by a reduced growth rate beginning from early childhood, in the absence of LWD (40). Here, it is noteworthy that 45,X is associated with gross chromosome imbalance, since chromosome imbalance, whether it is caused by the increase or decrease of chromosomal material, has been suggested to result in global developmental defects including growth failure (42, 43). Indeed, the adult height in patients with sex chromosome aberrations has been explained by the dosage effect of SHOX and the Y-specific growth gene and by the degree of growth disadvantage caused by chromosome imbalance (2). Although 45,X Turner patients usually have gonadal dysgenesis, gonadal estrogen deficiency is unlikely to have a major effect on the adult height or childhood growth pattern: 1) the mean adult height is comparable between normal females and patients with 46,XX gonadal dysgenesis and between patients with testicular feminization syndrome and those with 46,XY gonadal dysgenesis (2); and 2) childhood growth is usually normal in patients with gonadotropin deficiency (44). Thus, it is inferred that the remaining growth deficit and the reduced growth rate from early childhood in 45,X Turner syndrome seems to be due to chromosome imbalance, although the possibility that an X-specific growth gene escaping X-inactivation might be present on Xp has not been excluded formally (such an X-specific growth gene escaping X-inactivation is absent from Xq) (2, 27).

Remarks and Conclusion

Several points should be made with regard to the present study. First, the analyzed patients are small in number. Second, a selection bias exists in the ascertainment of patients. Third, several patients, especially those with sex chromosome aberrations, might have cryptic mosaicism or tissue-specific mosaicism. Fourth, assessment of several skeletal stigmata is more or less subjective. Lastly, the effect of SHOX haploinsufficiency may be somewhat modified in several patients by coexisting chromosomal aberration.

Despite the above caveats, the present study provides a useful clue for the elucidation of clinical features in SHOX haploinsufficiency. We propose that haploinsufficiency of SHOX is associated with not only short stature and LWD but also limb skeletal anomalies such as short 4th metacarpals and cubitus valgus, and that gonadal estrogens play an important role in the development of skeletal lesions and in the definition of growth patterns. This notion awaits additional studies such as investigation of a large number of patients with SHOX haploinsufficiency, identification of intragenic mutations of SHOX in patients with short 4th metacarpals and/or cubitus valgus, and examination of the relationship between skeletal lesions and gonadal function in patients with sex chromosome aberrations.

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