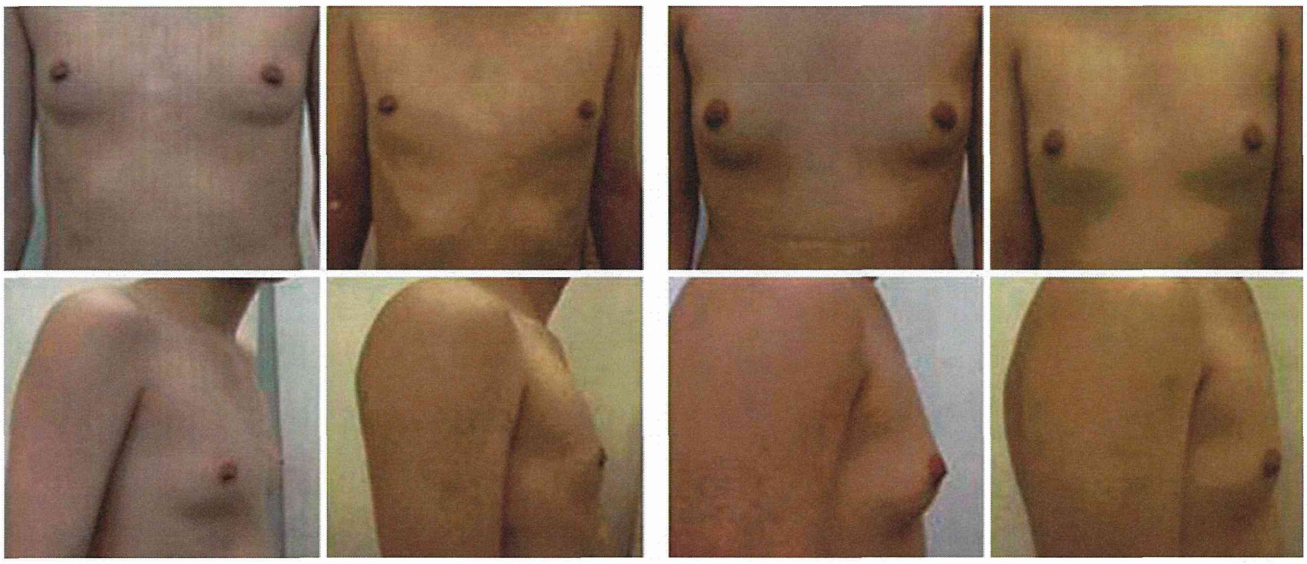

Fig. 1. Physical appearances of cases 1 (**a**) and 2 (**b**). Cases 1 and 2 presented with gynecomastia of Tanner stage 3. Tamoxifen treatment ameliorated gynecomastia to Tanner stage 2.

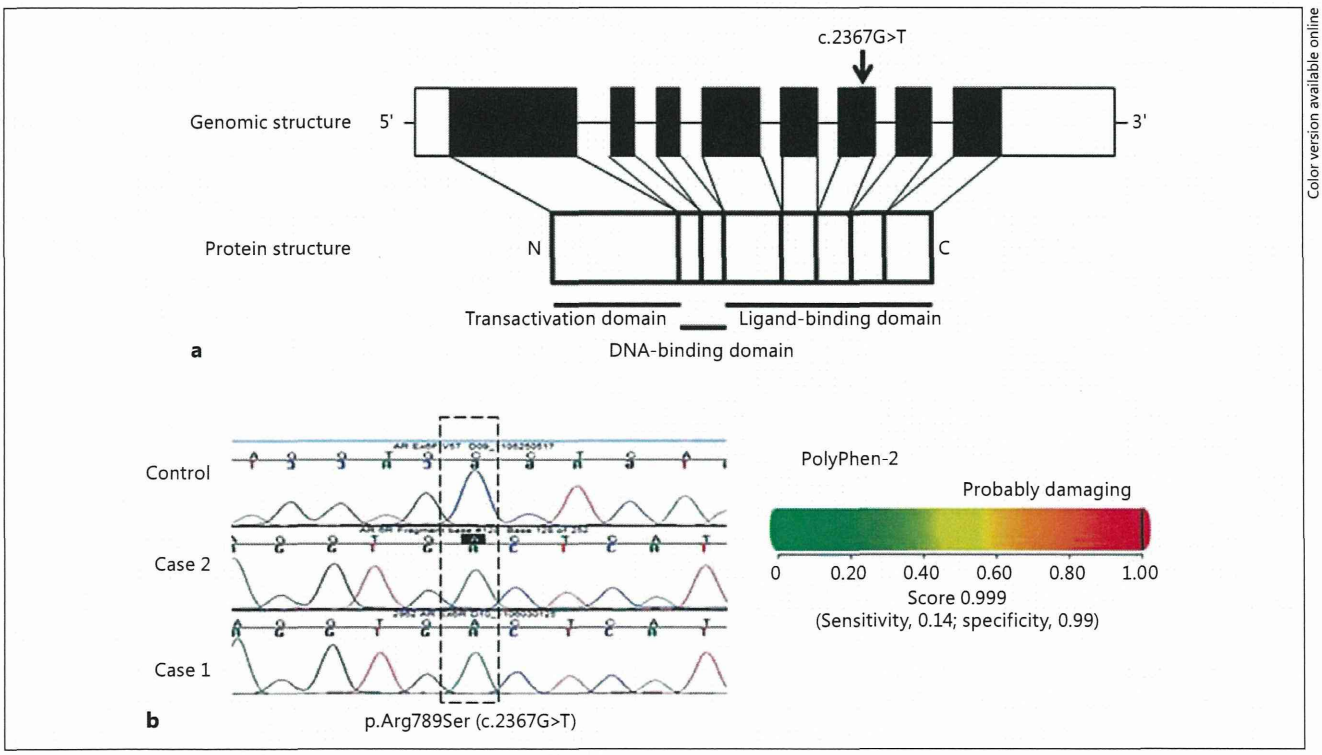

Fig. 2. Structure of the *AR* and the mutation identified in the present study. **a** Genomic and protein structures of *AR*. In the genomic structure, the white and black boxes indicate the noncoding and coding exons, respectively. The position of the nucleotide alteration (c.2367G>T) is indicated. **b** The results of direct sequencing (left panel, reverse sequences are shown) and in silico prediction analysis using PolyPhen-2 (right panel).

Table 1. Hormone values, clinical findings and treatment in cases 1 and 2

	Case 1					Case 2									Reference range[a]	
Age, years:months	13:00	13:03	14:02	14:04		15:09	16:07	16:00		16:02	16:03	16:05	16:10	17:07		
Hormone values	B	B	B	B	S[b]	B	B	B	S[b]			B	B	B	B	S[b]
LH, IU/l	3.7	**9.0**	5.1	**18.7**	**72.4**	**33.5**	**31.7**	5.5	20.1	...	...	**18.4**	**17.0**	**15.4**	2.9–8.2	18.2–38.0
FSH, IU/l	6.4	**12.2**	9.2	**20.2**	**26.8**	**13.9**	**12.3**	1.3	1.9	...	...	6.0	9.6	3.0	2.9–8.2	5.8–22.3
Testosterone, nmol/l	23.2	**51.0**	**47.5**	**114.4**		**69.7**	**74.5**	**36.8**		...	...	**46.8**	**49.9**	**53.7**	6.9–26.3	
Estradiol, pmol/l	<37	140	106	**419**		**345**	**275**	158		...	...	**389**	**297**	**294**	73–217	
TSH, mU/l	4.8		1.8					3.8							0.3–6.5	
free T4, pmol/l	11.6		16.7					14.2							11.6–21.9	
Prolactin, mU/l	578		417												152–557	
Clinical findings																
Breast (Tanner stage)	**3**	**2**	**3**	**2**		**2**	**2**	**3**		**3**	**2**	**2**	**2**	**2**		
Pubic hair (Tanner stage)	3			3		4	4	3					4	4		
Testis, ml	8			10		**10**	**10**	**10**					20	20		
Penile length (SD)	−0.7					**−3.0**	**−2.1**	−1.8					−1.8	**−3.3**		
Treatment																
Tamoxifen, mg/day	0→20[c]	20→0[c]	0→20[d]	20→40[e]		40	40			0→20[d]	20→40[e]	40	40	40		
TE, mg/month	...	...	...	...		0→150[f]	150			...	...	...	...	0→150[f]		

Hormone values below the reference range are underlined, and those above the reference range are boldfaced. Abnormal clinical findings are boldfaced. B = Basal; LH = luteinizing hormone; S = stimulated; TSH = thyroid-stimulating hormone. [a] Reference ranges for age-matched Japanese boys. [b] GnRH stimulation tests (100 μg bolus i.v.; blood sampling at 0, 30, 60, 90, and 120 min). [c] Tamoxifen was started at 13 years of age and discontinued after 3 months. [d] Tamoxifen was started at a dose of 20 mg/day. [e] Dose of tamoxifen was increased to 40 mg/day. [f] TE was started at a dose of 150 mg/month i.m.

Clinical Course

The clinical courses of cases 1 and 2 are shown in figure 1 and table 1. Since cases 1 and 2 experienced severe psychological distress due to gynecomastia, we initiated oral tamoxifen treatment (20 mg/day). Breast enlargement in both cases ameliorated from Tanner stage 3 to stage 2 after 2 months of tamoxifen treatment with no adverse events. After 3 months, the dose was increased to 40 mg/day. Since cases 1 and 2 were molecularly diagnosed with PAIS at 15.9 and 17.7 years of age, respectively, additional treatment with testosterone enanthate (TE; intramuscular injection, 150 mg/monthly) was started for correction of the mildly hypomasculinized genitalia and scarce body hair. TE treatment for 5 months induced laryngeal prominence and acne in the 2 cases; however, it caused no remarkable changes in penile length or body hair.

Hormonal Findings

The endocrine findings of cases 1 and 2 are summarized in table 1. Before tamoxifen therapy, their blood hormone levels were within the normal range, except for decreased estradiol levels in case 1, and decreased follicle-stimulating hormone (FSH) and slightly increased testosterone levels in case 2. During tamoxifen treatment, the blood levels of luteinizing hormone, testosterone, and estradiol were markedly elevated in both cases. In addition, the basal and GnRH-stimulated levels of FSH during treatment were significantly elevated in case 1, while they remained within the normal range in case 2. Additional treatment with TE caused no remarkable changes in the hormone values of case 1. Endocrine data for case 2 under TE treatment were not available.

Discussion

We identified a p.Arg789Ser mutation in *AR* in 2 siblings with persistent pubertal gynecomastia and various symptoms of mild hypomasculinization. The phenotypes of cases 1 and 2 could be ascribed to this mutation because in silico analysis suggested p.Arg789Ser as a probable damaging mutation. In fact, p.Arg789Ser has previously been detected in a patient with gynecomastia, a high-pitched voice, and decreased body hair [15], and in a patient with gynecomastia and ambiguous genitalia [16]. These findings support the notion that persistent pubertal gynecomastia is an essential feature in patients with hypomorphic mutations in *AR* [12].

Tamoxifen treatment for 2 months significantly ameliorated gynecomastia in the 2 cases. These results indicate that in the breast tissue, the antiestrogenic effect of tamoxifen can counterbalance the impaired androgen action caused by *AR* mutations. In this regard, it is worth mentioning that SERMs have been used for a number of patients with idiopathic pubertal gynecomastia [3, 8, 9]. Although other therapeutic options, such as aromatase inhibitor therapy and surgery have also been used for idiopathic pubertal gynecomastia, the response rate to therapy with SERMs (84–90%) is significantly higher than that to aromatase inhibitor therapy (almost equivalent to placebo) [2, 4, 8]. Previous case series have reported success with tamoxifen at a dosage of 20–40 mg daily for 2–9 months for idiopathic pubertal gynecomastia and few recurrences of gynecomastia after cessation of therapy [2, 4]; however, the tamoxifen dose and duration for the PAIS patients is unclear. Future studies are necessary to clarify the recommended dose and duration of tamoxifen treatment for PAIS patients.

When gynecomastia is present for more than 1 year, the tissue becomes inactive and fibrotic [2]. In our 2 cases, tamoxifen treatment showed a significant effect in amelioration of gynecomastia within 2 months; however, mild gynecomastia still remained. This may be caused by progress to dense fibrosis. Furthermore, while SERMs are rarely associated with adverse events in adolescent boys [8, 9], surgery is known to result in various minor complications in more than 50% of patients [9]. Therefore, SERMs are considered to be useful for idiopathic pubertal gynecomastia.

The present study shows for the first time that tamoxifen can also ameliorate pubertal gynecomastia associated with PAIS. Our results, when considered together with significantly improved spermatogenesis after tamoxifen treatment in an adult patient with PAIS [17], imply that SERMs may provide considerable benefit to patients with PAIS. In this context, androgen supplementation therapy may be considered to be more helpful than SERMs for patients with PAIS because androgens appear to improve not only gynecomastia but also genital abnormalities [11, 12]. However, the effect of androgens may vary among patients depending on the functional activities of the mutant AR proteins [10, 11]. Indeed, TE treatment induced only minor responses in our cases. Thus, further studies are necessary to clarify the effects of SERMs and androgens in patients with PAIS.

The endocrine evaluation of cases 1 and 2 provided notable findings. Both cases had almost normal hormone values before tamoxifen treatment, and significantly increased levels of luteinizing hormone, testosterone and estradiol during treatment. These hormone data are almost comparable to those in previously reported untreated patients with PAIS [12]. Furthermore, markedly elevated FSH levels similar to those in case 1 have been reported in several patients with PAIS [12]. Such hormone alterations can be explained by perturbed feedback regulation of gonadotropins resulting from impaired AR function in the brain [10, 11]. However, since tamoxifen is known to exert a stimulatory effect on the hypothalamus-pituitary-gonadal axis in normal males [18], it remains possible that tamoxifen played a role in the hormone abnormalities of our cases.

In summary, our findings indicate that tamoxifen is effective in treating pubertal gynecomastia in patients with PAIS. These results provide a novel therapeutic option for gynecomastia associated with endocrine disorders. Further studies that compare the effects of SERMs and androgens, along with longitudinal monitoring for hormone levels, are necessary to establish an optimal management protocol for gynecomastia in patients with PAIS.

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Disclosure Statement

The authors have nothing to disclose.

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Review

Skeletal Deformity Associated with *SHOX* Deficiency

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Abstract. *SHOX* haploinsufficiency due to mutations in the coding exons or microdeletions involving the coding exons and/or the enhancer regions accounts for approximately 80% and 2–16% of genetic causes of Leri-Weill dyschondrosteosis and idiopathic short stature, respectively. The most characteristic feature in patients with *SHOX* deficiency is Madelung deformity, a cluster of anatomical changes in the wrist that can be attributed to premature epiphyseal fusion of the distal radius. Computed tomography of SHOX-deficient patients revealed a thin bone cortex and an enlarged total bone area at the diaphysis of the radius, while histopathological analyses showed a disrupted columnar arrangement of chondrocytes and an expanded hypertrophic layer of the growth plate. Recent studies have suggested that perturbed programmed cell death of hypertrophic chondrocytes may underlie the skeletal changes related to *SHOX* deficiency. Furthermore, the formation of an aberrant ligament tethering the lunate and radius has been implicated in the development of Madelung deformity. Blood estrogen levels and mutation types have been proposed as phenotypic determinants of *SHOX* deficiency, although other unknown factors may also affect clinical severity of this entity.

Key words: chondrocyte, Leri-Weill dyschondrosteosis, Madelung deformity, short stature, Vickers ligament

Introduction

SHOX (NM_000451.3) encodes a transcription factor exclusively expressed in the developing limb and pharyngeal arch in the human embryo (1). Heterozygous mutations of *SHOX* lead to Leri-Weill dyschondrosteosis characterized by wrist deformity and mesomelic short stature (LWD; OMIM #249700), as well as idiopathic short stature without apparent skeletal malformations (ISS; OMIM # 300582) (2–4). Less specific skeletal changes such as high arched palate, short metacarpals, scoliosis, and micrognathia have also been described in patients with *SHOX* deficiency (5). Previous studies have shown that *SHOX* deficiency accounts for approximately 80% and 2–16% of genetic causes of LWD and ISS, respectively (5–8). Furthermore, haploinsufficiency of *SHOX* represents the major cause of growth failure in patients with Turner syndrome (9). Thus, *SHOX* deficiency is a clinically important condition,

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particularly in the field of pediatric endocrinology and orthopedics. This review article introduces our current understanding of the causative mechanisms and phenotypic characteristics of SHOX-associated skeletal malformations.

Molecular Basis of *SHOX* Deficiency

SHOX resides in the short arm pseudoautosomal region of the X and Y chromosomes (PAR1) and escapes X inactivation (2). Thus, although *SHOX* is located on the sex chromosomes, molecular defects of *SHOX* are inherited in an autosomal dominant manner. To date, several point mutations in the *SHOX*-coding exons and submicroscopic deletions encompassing the coding region and/or the upstream or downstream enhancer regions have been identified in more than 200 patients with LWD or ISS (5–9). Previously reported *SHOX* mutations are listed in the SHOX Mutation Database (http://hyg-serv-01.hyg.uni-heidelberg.de/lovd/index.php?select_db=SHOX) (10). The precise position of the *SHOX* enhancers remains to be determined, although the upstream and downstream enhancers have been mapped to a ~300 kb region ~95 kb upstream and an ~30 kb region ~250 kb downstream from the start codon, respectively (11–13). Deletions involving the coding and/or downstream enhancer regions account for about 60% of Japanese LWD patients (14), and those affecting the downstream enhancer regions are the major genetic causes in Spanish patients (15). High recombination frequency and the abundant presence of repeat sequences in PAR1 likely play a role in the development of submicroscopic genomic rearrangements involving *SHOX* (14).

Skeletal Deformity in Patients with *SHOX* Deficiency

The most characteristic feature in patients with *SHOX* deficiency is Madelung deformity, a cluster of anatomical changes in the forearm including bowing and shortening of the radius, prominence of the ulnar head and palmar and ulnar deviation ("pyramidal configuration") of the carpal bones (Fig. 1 and Fig. 2) (16). Clinical manifestations induced by Madelung deformity include wrist pain, deformation and limited joint motion (16, 17). Radiological findings of Madelung deformity include the absence or narrowing of the ulnar portion of the distal radial physis, anterior bowing of the radial shaft and dorsal subluxation of the ulnar head (17, 18). Several additional skeletal changes such as triangularization of the distal radial epiphysis have been associated with

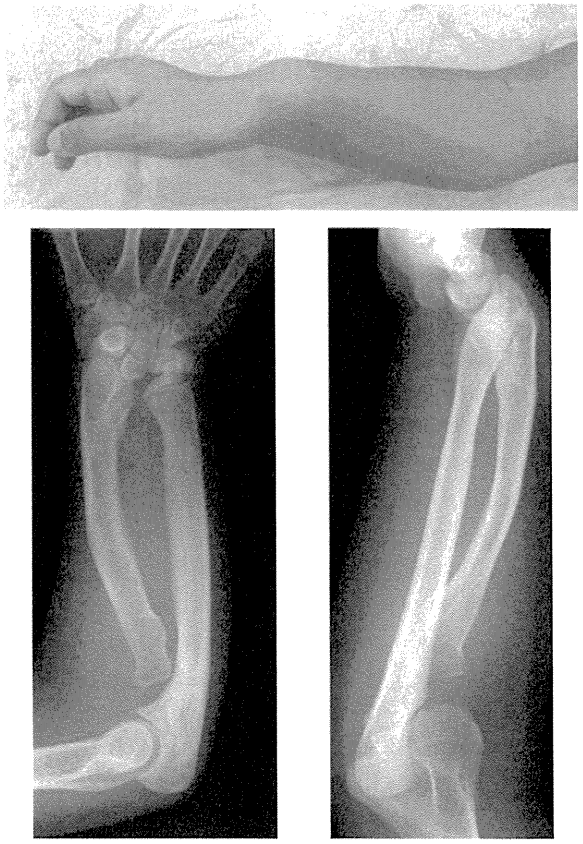

Fig. 1. Madelung deformity in a female patient with *SHOX* deficiency. Upper panel: appearance of the forearm. Prominence of the distal ulna is shown. Lower panel: radiographic findings. Shortening and bowing of the radius and dorsal subluxation of the ulnar head are shown.

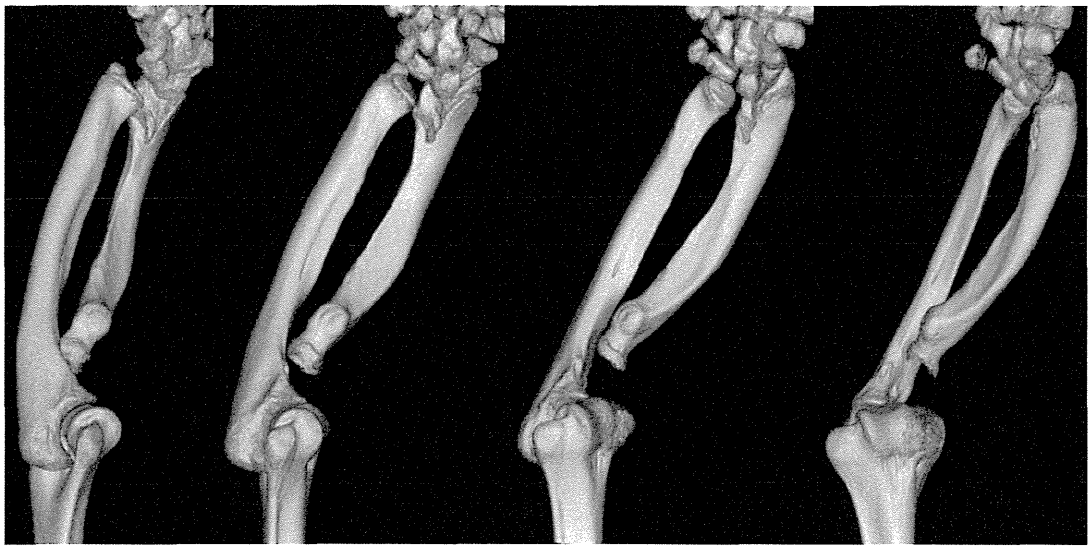

Fig. 2. Forearm three-dimensional computed tomography of a female patient with *SHOX* deficiency. Significant findings include shortening of the radius, pyramidal configuration of the carpal bones and dorsal subluxation of the ulna in addition to severely disturbed structural organization of the elbow joint.

Madelung deformity (19). In patients with severe Madelung deformity, the structural organization of the elbow joint is also disrupted (Fig. 2).

Previous studies have suggested that the primary lesion of Madelung deformity is the premature epiphyseal fusion at the volar-ulnar portion of the radial growth plate (20). Impaired growth of the radius due to the early epiphyseal fusion, in combination with relatively preserved growth of the ulna, appears to underlie the characteristic deformity (18, 21). The appearance of the wrist varies among patients and probably depends on the fusion position along the anterior-posterior axis of the radial epiphysis (22).

Although Madelung deformity usually occurs as a result of *SHOX* deficiency, it can also take place as a component of other congenital disorders such as multiple exostoses syndrome, multiple epiphyseal dysplasia and dysostosis multiplex of mucopolysaccharidosis (23). Madelung-like deformity has also been observed in patients with pseudohypoparathyroidism type 1b (24). In addition, Madelung deformity can occur as a change secondary to injury and infection (19).

When Madelung deformity is accompanied by short stature and mesomelic shorting of the limbs, it is referred to as LWD. The mesomelic short stature of LWD can be explained as a result of impaired linear growth of the radius, ulna, tibia and fibula. A decreased extremity/trunk ratio with a fairly preserved sitting height and head circumference is a characteristic auxological finding of patients with LWD (5, 25). Rappold et al. developed a phenotype scoring system for screening of individuals with possible *SHOX* deficiency from patients with short stature (8). They suggested the following eight clinical features as indicators for *SHOX* deficiency: arm span/height ratio, sitting height/height ratio, body mass index, cubitus valgus, short forearm, bowing of the forearm, muscular hypertrophy and dislocation of the ulna. Although *SHOX* deficiency is the only condition that has been implicated in LWD, *SHOX* abnormalities have been detected only in 50–90% of patients with LWD (5). It remains currently unknown whether LWD patients with apparently normal *SHOX* alleles have mutations in the regulatory regions

of *SHOX* or in a hitherto unidentified gene involved in skeletal development.

Changes in Bone Geometry and Bone Mineral Density

Using peripheral quantitative computed tomography of the forearm, Soucek *et al.* investigated bone mineral density and bone geometry in 10 prepubertal patients with *SHOX* deficiency and 22 patients with Turner syndrome (26). They found that patients of both groups had a thin bone cortex and an enlarged total bone area at the diaphysis of the radius compared with control individuals. On the other hand, these patients had a normal trabecular bone mineral density and bone strength index. Soucek *et al.* proposed that the skeletal changes observed in patients with *SHOX* deficiency are attributable to an adjustment of the long bones with a disrupted cortex to the mechanical loading that aims to increase bone strength.

Histopathological Changes

Munns *et al.* investigated histopathological findings of the surgically-excised growth plate of the distal radius obtained from two patients with molecularly confirmed *SHOX* deficiency (22). They found disrupted columnar arrangement of chondrocytes; the normal tandem stacking of mature chondrocytes within columns was replaced by a side-by-side arrangement. Furthermore, the presence of hypertrophic osteoid with micro-enchondromata in the metaphysis suggested aberrant endochondral ossification. Significant expansion of the hypertrophic layer and reduction of the proliferative layer were observed in the growth plate. These data imply that the SHOX protein is required for ordered zonal development of chondrocytes. In this regard, *SHOX* is strongly expressed in terminally differentiated hypertrophic chondrocytes and less obviously in proliferating and reserve chondrocytes (27). *In vitro* assays with osteosarcoma cells indicated that SHOX induces oxidative stress and activates the intrinsic apoptotic pathway (27). Thus, it is possible that SHOX plays a critical role in chondrocyte development by regulating the cell cycle and apoptosis of hypertrophic chondrocytes. Indeed, premature epiphyseal fusion in patients with *SHOX* deficiency may reflect a perturbed cell death process in the growth plate. To date, however, the precise mechanism by which SHOX exerts its effect on chondrocyte development remains unknown. Although *in vivo* and *in vitro* assays have indicated that several proteins such as BNP, FGFR3, SOX5, and SOX6 can interact with SHOX (28, 29), the function of SHOX in human tissues has yet to be elucidated.

Formation of an Abnormal Ligament

Vickers and Nielsen identified an abnormal ligament in patients with Madelung deformity (30). The "Vickers ligament" tethers the lunate to the distal portion of the radius and can have a diameter as large as 8 mm (Fig. 3) (22). Histological analysis demonstrated that the Vickers ligament is a morphologically normal ligament consisting of collagen and elastin fibers (22). This ligament is predicted to promote pyramidal configuration of the carpal bones by disturbing the physiological migration of these bones during growth (22). Furthermore, this ligament may exert an inhibitory effect on linear growth of the radius by compressing its distal epiphysis (18). Therefore, it is possible that the Vickers ligament constitutes an essential factor in the development of Madelung deformity. Although the process by which the Vickers ligament forms has yet to be clarified, this ligament is regarded as a secondary change of the forearm deformity (30, 31). Actually, the ligament seems to consist of hypertrophied connective tissues that form under a mechanical force that arises from asymmetrical growth of the radius and ulna.

Recent advancements in high-resolution magnetic resonance imaging have enabled early

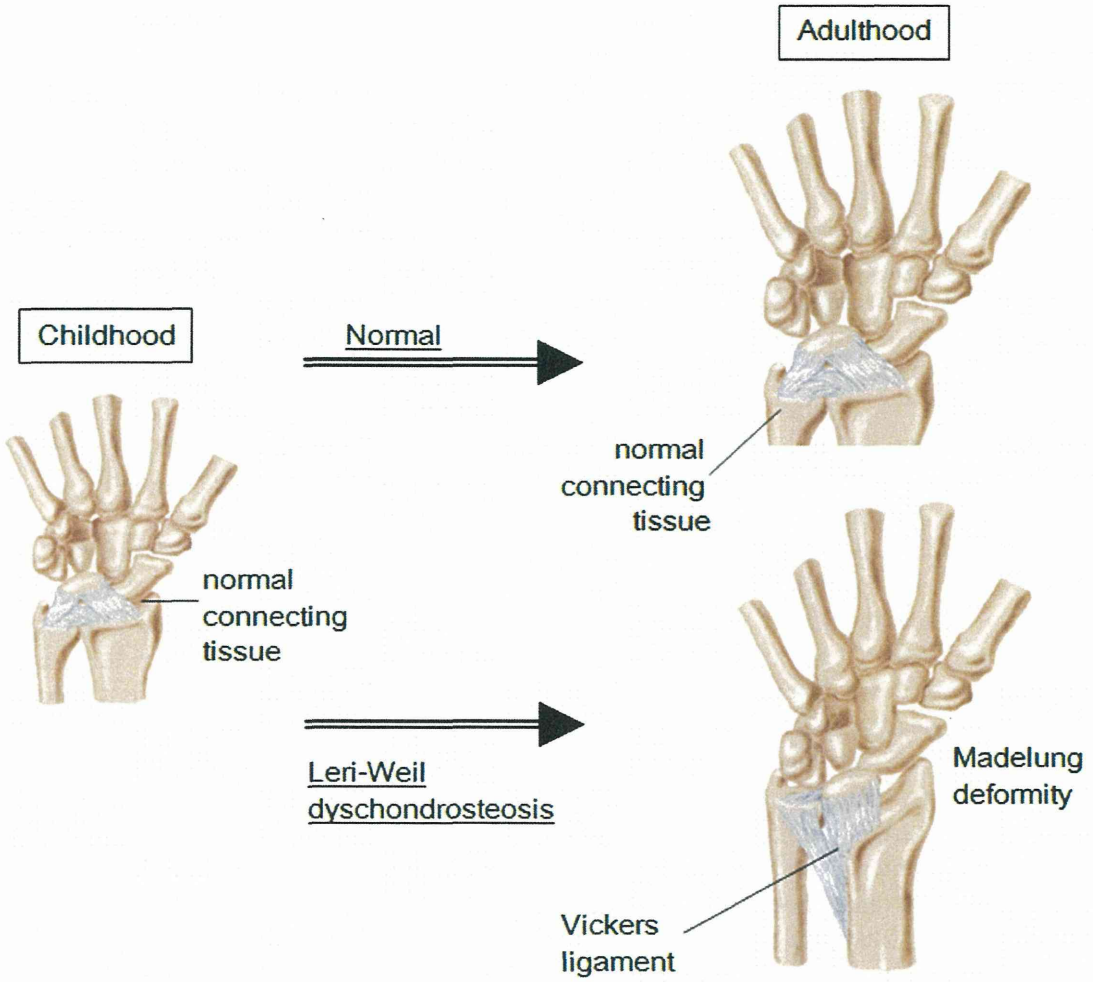

Fig. 3. Schematic representation of the Vickers ligament that tethers the lunate to the distal portion of the radius. This ligament seems to consist of hypertrophied connective tissues that form under the mechanical force that arises from asymmetrical growth of the radius and ulna.

detection of the Vickers ligament (31). Previous studies have indicated that surgical removal of the Vickers ligament in combination with dome osteotomy is beneficial to patients with Madelung deformity; Harley *et al.* reported that these surgical interventions effectively improved the clinical features of adolescent patients with Madelung deformity (21), while Steinman *et al.* showed that these interventions can provide long-term correction of the wrist deformity (16). However, since the number of treated patients was small, further studies are necessary to validate these findings.

Phenotypic Determinants

Skeletal changes of *SHOX* deficiency tend to be more severe in adult females than in children or adult males (5). Obvious Madelung deformity is rare in prepubertal patients, although decreased extremity/trunk ratios and subtle skeletal changes are observed in the majority

of children with *SHOX* deficiency (5, 32, 33). These data can be explained by assuming that estrogens exert a deleterious effect on skeletal formation in patients with *SHOX* abnormalities. Since estrogens induce physiological skeletal maturation in both sexes (34), they may also enhance premature epiphyseal fusion in patients with *SHOX* deficiency. Consistent with this, severe Madelung deformity is rarely seen in Turner females in whom ovarian function is frequently impaired (35). Furthermore, a longitudinal study of a female patient with *SHOX* deficiency and normal ovarian function showed age-appropriate skeletal maturation before puberty and rapidly advanced bone age during puberty (36). On the other hand, since Soucek et al. revealed a significant difference in bone geometry between prepubertal patients with the 46,XX karyotype and prepubertal Turner females, it is likely that some factors other than estrogens may also underlie relatively mild skeletal features in Turner females (26). Soucek et al. suggested karyotype mosaicism as one of the possible candidates for such factors (26). It is known that the karyotype of Turner females is heterogeneous and includes 45,X/46,XX. Since two normal *SHOX* alleles are present in a certain percentage of cells in females with the 45,X/46,XX karyotype, this may lead to relatively well preserved skeletal structures in such patients.

Mutation types may affect the phenotypic severity of *SHOX* deficiency. It has been proposed that molecular defects involving only the enhancer regions are associated with broader phenotypic variation than deletions/mutations affecting the coding exons; Chen et al. have described more severe skeletal changes in patients with enhancer deletions than in those with mutations/deletions affecting the coding exons (37), while Rosilio et al. reported relatively mild phenotypes in patients with enhancer deletions (7). On the other hand, no apparent genotype-phenotype correlation has been reported for *SHOX* intragenic mutations/deletions (8).

Conclusion

Recent studies have indicated that *SHOX* deficiency leads to premature epiphyseal fusion at the distal radius, possibly by disturbing programmed cell death of hypertrophic chondrocytes. In addition, the formation of an aberrant ligament tethering the lunate and radius appears to play a role in the development of Madelung deformity. Blood estrogen levels and mutation types have been proposed as phenotypic determinants of *SHOX* deficiency, although other unknown factors may also modify the clinical severity of this condition.

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