SHOX duplications found in some cases with type I Mayer-Rokitansky-Kuster-Hauser syndrome

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Purpose: The Mayer-Rokitansky-Küster-Hauser syndrome is defined as congenital aplasia of müllerian ducts derived structures in females with a normal female chromosomal and gonadal sex. Most cases with Mayer-Rokitansky-Küster-Hauser syndrome are sporadic, although familial cases have been reported. The genetic basis of Mayer-Rokitansky-Küster-Hauser syndrome is largely unknown and seems heterogeneous, and a small number of cases were found to have mutations in the *WNT4* gene. The aim of this study was to identify possible recurrent submicroscopic imbalances in a cohort of familial and sporadic cases with Mayer-Rokitansky-Küster-Hauser syndrome. **Methods:** Multiplex ligation-dependent probe amplification was used to screen the subtelomeric sequences of all chromosomes in 30 patients with Mayer-Rokitansky-Küster-Hauser syndrome (sporadic, $n = 27$ and familial, $n = 3$). Segregation analysis and pyrosequencing were applied to validate the MLPA results in the informative family. **Results:** Partial duplication of the Xpter pseudoautosomal region 1 containing the short stature homeobox (*SHOX*) gene was detected in five patients with Mayer-Rokitansky-Küster-Hauser syndrome (familial, $n = 3$ and sporadic, $n = 2$) and not in 53 healthy controls. The duplications were not overlapping, and *SHOX* was never entirely duplicated. Haplotyping in the informative family revealed that *SHOX* gene duplication was inherited from the unaffected father and was absent in two healthy sisters. **Conclusions:** Partial duplication of *SHOX* gene is found in some cases with both familial and sporadic Mayer-Rokitansky-Küster-Hauser type I syndrome. ***Genet Med* 2010:12(10):634–640.**

Key Words: *MRKH, SHOX, MLPA, PAR1, müllerian aplasia*

The müllerian ducts are the anlage of the female reproductive tract. They differentiate to form the Fallopian tubes, the uterus, the uterine cervix, and the upper vagina. Disruption of the morphogenetic process results in a wide variety of malformations. Complete müllerian aplasia or hypoplasia is known as Mayer-Rokitansky-Küster-Hauser (MRKH) syndrome (OMIM #277000). This disorder is characterized by malformations of the structures derived from the müllerian ducts. MRKH syndrome is the second most common cause of primary amenorrhoea and has an incidence of approximately 1 in 5000 female live births.[1]

MRKH syndrome may occur as an isolated abnormality (MRKH type 1 syndrome). However, in around 15–40% of the cases it is associated with some skeletal or urological malformations (MRKH type II syndrome), including (i) the Klippel-Feil syndrome (OMIM #118100, #214300),[2] characterized by congenital fusion of the cervical spine, short neck, low posterior hairline, and limited motion of the cervical spine and (ii) müllerian aplasia, renal aplasia, and cervicothoracic somite dysplasia (MURCS; OMIM #601076). Rarely associated abnormalities include auditory and/or cardiac defects.[3,4] This wide spectrum of associated anomalies suggests the involvement of structures derived from the intermediate mesoderm including kidneys and female internal genitalia. Patients with MRKH syndrome have a normal 46,XX karyotype, although rare X chromosomal abnormalities have been reported in association with gonadal dysgenesis.[5–12]

Although most cases are sporadic, a number of familial cases have been described, raising the possibility that a subset of patients with MRKH syndrome are the result of a single-gene disorder, as reviewed by Guerrier et al.[13]

Shokeir[14] investigated 10 families with several members affected by MRKH syndrome. In the majority of them (8/10), there were some affected paternal relatives, raising the possibility of an autosomal dominant inheritance with sex-limited (female) expression and incomplete penetrance. It was suggested that female carriers develop müllerian abnormalities, whereas male carriers do not manifest any deleterious effect.[14]

The genetic basis of MRKH syndrome is largely unknown. Array-comparative genomic hybridization (CGH) analyses have detected submicroscopic imbalances at 1q21.1, 4q34-qter, 17q12, 22q11.21, and Xq21.31.[15,16] Recurrent changes have also been identified at 22q11q21.1 and 17q12.[1,15–17] However, analysis of candidate genes have been inconclusive.[10,13,15] Investigated loci include antimüllerian hormone and its receptor *CTNNB1*[18,19] and genes involved in early development such as *WT1*,[20] *PAX2*,[20] *HOX*,[21,22] *LMX1,* and *TCF2*.[15] Mutations in the *WNT4* gene have been found in four unrelated patients with MRKH syndrome with clinical signs of hyperandrogenism[23–25] (OMIM #158330), suggesting that MRKH syndrome with hyperandrogenism may be a clinical and genetic distinct disorder. However, given the low number of identified cases, it is unlikely that *WNT4* plays a major role in the etiology of MRKH syndrome.

Taken together, these results suggest that MRKH syndrome is a clinically and genetically heterogeneous disorder.

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This report describes the molecular genetic investigation of a cohort of 30 women presenting with MRKH type I or type II syndrome. Our cohort included one family in which two of four sisters were affected by MRKH type I syndrome. This family was investigated using multiplex-ligation-dependent-probe-amplification (MLPA) to screen for imbalances at the subtelomeric regions of autosomes and sex chromosomes. A partial duplication of the gene *SHOX* was detected in the two probands and their father. The analysis was, therefore, extended to include other 28 patients with MRKH syndrome of the cohort.

The *SHOX* gene is a member of the paired homeobox family and is located in the pseudoautosomal region 1 (PAR1) of chromosomes X and Y. *SHOX* controls fundamental aspects of growth and development, and its mutations have been associated with three distinct phenotypes: (i) Leri-Weill Dyschondrosteosis (OMIM #127300), a dominantly inherited skeletal dysplasia characterized by moderately short stature secondary to short mesomelic limb segments and Madelung wrist deformities; (ii) Langer Mesomelic Dysplasia (OMIM #249700), a rare recessive skeletal dysplasia characterized by extremely short stature secondary to shortening and malformation of the mesomelic and rhizomelic segments of the limbs; and (iii) idiopathic short stature (OMIM #300582[26]).

The results of this study suggest that the *SHOX* gene is also implicated in both familial and sporadic type I MRKH syndrome.

PATIENTS AND METHODS

With regard to human subjects, we followed the guidelines of the ethical committee of the University of Milan (http://www.unimi.it/cataloghi/comitato_etico/CE_Rec_4__HBMs.pdf).

Table 1. Clinical data of patients with MRKH syndrome

Enrolled patients	30
Mean age at diagnosis (yr)	22
MRKH type I	15
MRKH type II	14
MURCS	1
Mean weight (kg)	55
Mean height (cm)	161
Mean BMI	21
Mean head circumference (cm)	55
Patients with hyperandrogenism	12

Patients

A total of 30 patients with MRKH syndrome were enrolled in the study after informed consent. The cohort included an affected sib-pair and one patient with a family history positive for MRKH syndrome. The other 27 patients were sporadic patients. Diagnostic criteria for MRKH syndrome included normal external genitalia, presence of pubic and axillary hair, absence of a vagina, presence of mullerian remnants, and no cystic swelling secondary to retained menstrual blood. The following auxologic data were collected: height, weight, body mass index (BMI), head circumference, arm span, span to height ratio, hand length, middle finger length, foot length, internal and external interchantal distance, and ear length. The relevant clinical data are summarized in Table 1. Neither severe abnormalities nor dysmorphic features were observed. All patients underwent ultrasonography of the pelvis and urinary system, pelvic magnetic resonance imaging, and karyotyping. Patients were classified as having type I or type II MRKH syndrome according to the absence or presence of other genitourinary malformations. Type II patients included (i) MURCS and (ii) MRKH syndrome associated with other clinical findings.[4] Mean age at diagnosis was 22 years (range: 16–33 years). The patients showed no evidence of cognitive dysfunction.

Familial patients

Family 1. Family 1 was composed of six members. The parents were healthy and nonconsanguineous. Two paternal aunts were reported to be infertile. However, they were not available for the study, and no additional information was available. Two of the four daughters had MRKH type I syndrome (Patients II-3 and II-4). At inclusion into the study, one sister was underweight (BMI = 16.6), and the other was severely underweight (BMI = 13.3) (Table 2). All other parameters, including height and head circumference, were within the normal range. The affected sisters and their father had a normal karyotype.

Family 2. Family 2 was composed of four members. The parents were healthy and nonconsanguineous. Both daughters had MRKH type I syndrome. One sister refused to participate in the study. The auxologic and morphologic data of the investigated sister were within the normal range (Table 2). The patient showed clinical signs of hyperandrogenism (hirsutism and acne) and had a normal karyotype.

Sporadic patients

This cohort included 27 women who had been referred for management of primary amenorrhea. Twenty-six patients were Italians, and one was Filipino. Twelve patients were classified as having MRKH type I syndrome (Table 1). The remaining 15 patients were classified as having MRKH type II syndrome

Table 2. Diagnosis and main clinical features of patients with MRKH type 1 syndrome heterozygous for partial *SHOX* gene duplications

Patients	Age at diagnosis (yr)	Weight (kg)	Height (cm)	BMI	Head circumference (cm)	Hyperandrogenism
1	19	49	1.65	14.8	55	No
2	30	44	1.54	14.3	53	Yes
3	29	56	1.68	16.7	57	Yes
II-3	25	42	1.58	13.3	54	No
II-4	23	53	1.60	16.6	58	No

(MURCS $n = 1$, Table 1; MRKH syndrome with additional clinical findings $n = 14$). Twenty-four percent of the patients had skeletal defects and 14% urinary tract malformations. Eleven patients showed clinical features of hyperandrogenism. All patients had a normal female karyotype (46,XX).

Six patients reported a positive family history of dysmorphism, malformations, or mental retardation. In the remaining patients, the family history was unremarkable.

Molecular analysis

Genomic DNA was extracted from blood lymphocytes using the QIAamp DNA Mini Kit (Qiagen, Inc., Chatsworth, CA) in accordance with the manufacturer's instructions. Genomic DNA was obtained for all 30 patients, 53 healthy controls, and the parents from Family 1.

WNT4 mutational analysis

Patients with signs of hyperandrogenism ($n = 12$) were analyzed by direct sequencing of all *WNT4* gene exons using polymerase chain reaction (PCR) primers, as reported previously.[24]

MLPA analysis

MLPA analysis was performed using two commercial kits (MRC-Holland, Amsterdam, NL): P036 and P070, specific for all subtelomeres; P018 *SHOX* probe mix containing probes for each exon of the *SHOX* gene and several probes for regions upstream and downstream of the *SHOX* gene that have been implicated in its transcriptional regulation. The P018 *SHOX* probe mix was used only in a selected number of patients. The kits were used in accordance with the manufacturer's instructions, as described previously.[27–29] Five normal DNA samples were included as references for the statistical analysis. Each patient was tested in parallel with a further wild-type sample and control samples from cytogenetically abnormal patients comprising autosome and sex chromosome aneuploidy. PCR products were separated using an ABIPRISM 3130 automatic sequencer and analyzed using GeneMapper software (Applied Biosystem, Foster City, CA). The MRC Coffalyser MLPA-DAT software was used for the normalization and statistical analysis of MLPA fragment data files, in accordance with the manufacturer's protocol. All results were confirmed by performing a second independent MLPA experiment.

DNA from 53 healthy and fertile females (a total of 106 X chromosomes) was also tested with the P018 *SHOX* kit. These were used as reference samples to evaluate the presence, location, and frequency of copy number polymorphisms detected by the MLPA probes in patients. Probe positions are indicated in Figure 1.

Haplotyping

Genotyping was performed in Family 1 using a panel of single-nucleotide polymorphisms (SNPs) located in the PAR1. These included four *SHOX* intragenic SNPs. The SNPs were selected using the NCBI database (available at: www.ncbi.nlm.nih.gov), and their positions are indicated in Figure 2. Loci and primers are available on request. PCR products were detected using an ABI PRISM 3130 sequencer (Applied Biosystem, Foster City, CA). Electropherogram analyses were performed using Sequence Navigator and ChromasPro software.

Pyrosequencing

To confirm *SHOX* duplications by the assessment of allelic imbalance, DNA from informative patients was analyzed using a PyroMark ID instrument (Biotage AB, Uppsala, Sweden). Loci and primers are available on request and their positions are shown in Figure 2A.

Data quantification was performed using PyroMark ID software v1.0.9 (Biotage AB). This calculates the ratio of each allele as a percentage. For each sample, allelic contribution values represented the mean of at least two independent PCR and Pyrosequencing analyses.

RESULTS

No *WNT4* mutations were identified in the 12 patients with clinical signs of hyperandrogenism.

Partial *SHOX* duplications were detected in five of the 30 patients with MRKH syndrome. These included the two affected sisters of Family 1, the available familial patient from Family 2, and two sporadic patients.

Molecular results of the cohort

In five patients with MRKH syndrome, subtelomeric MLPA analysis demonstrated changes in the Xp telomeric region. Refined analysis using the *SHOX*-specific P018 MLPA kit revealed differing partial *SHOX* gene duplications in three patients (one familial and two sporadic) compared with those identified in the affected sib-pair from Family 1. Figure 1 summarizes the duplicated regions identified in the familial and the sporadic patients.

Family 1

In Patients II-3 and II-4 (Family 1), probes 1148-L1331 and 3714-L0910, which were included in the two subtelomeric MLPA kits, showed values of >1.3. This result is compatible with the presence of a terminal Xp duplication. DNA from the parents and two healthy sisters (II-1 and II-2) was also analyzed. Normal range values (0.7–1.3) were obtained for DNAs from II-1 and II-2. A signal pattern of >1.3 was obtained for both probes for the paternal DNA (data not shown). The P018 kit was then used in all members of Family 1 to confirm and refine the imbalanced region because these probes mapped to the PAR1 at 0.57 and 0.50 Mb from the Xp telomere at different positions in the *SHOX* gene (Fig. 1). A signal pattern consistent with a duplication of exons 4, 5, and 6, involving probes 1148-L1331, 1149-L0910, 1150-L0911, 1151-L0708, and 1152-L0709 was detected in the two affected sisters and in the paternal DNA (Figs. 1 and 2). The size of the duplicated region was estimated at approximately 17 kb (Fig. 1), based on the position of probes displaying normal signals flanking the probes with abnormal signals.

Patient 1 (sporadic)

In Patient 1, a de novo duplication of the region marked by probes 1145-L0702 (*SHOX* exon 1) and 1146-L6220 (*SHOX* exon 2) was identified. The size of this duplication was approximately 7 kb (Fig. 1).

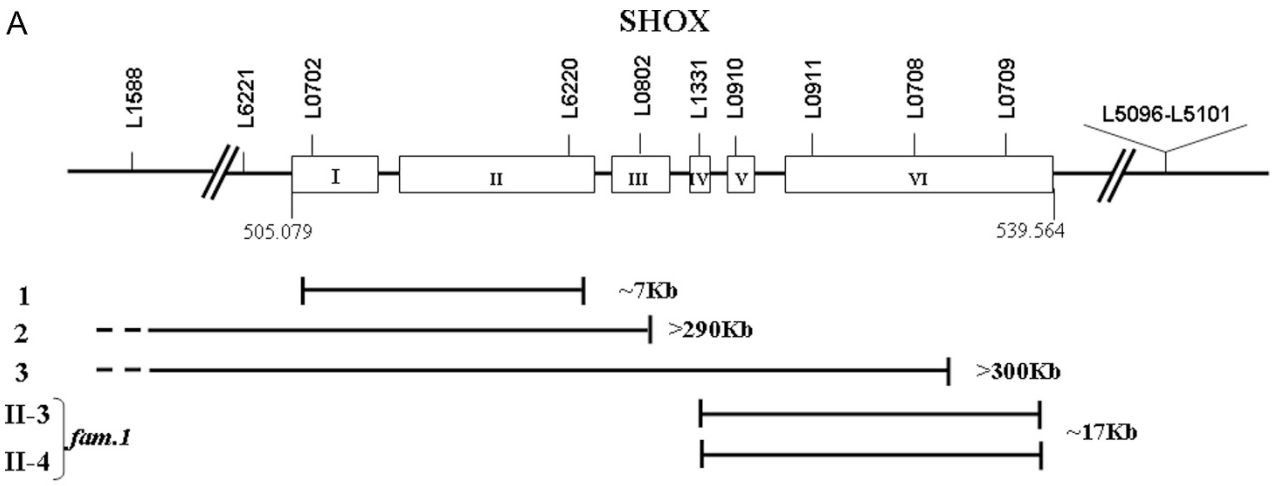
Patient 2 (Family 2)

In Patient 2, who was one of the affected daughters from Family 2, a duplication of >290 kb (Fig. 1), spanning between 2726-L1588 probe (located in *PPP2R3B*) and 1147-L0802 probe (in *SHOX* exon 3), was identified.

Patient 3 (sporadic)

In Patient 3, a duplication of >300 kb, spanning between 2726-L1588 probe (in *PPP2R3B*) and 1151-L0708 probe (located at the 3′ of the *SHOX* gene), was identified (Fig. 1). Because of the lack of MLPA probes telomeric to 2726-L1588,

A



B

			fam.1										
PROBE	probe position	physical map	I-1	I-2	II-1	II-2	II-3	II-4	1	2	3	45,X	47,XXY
2726-L1588	PPP2R3B	227444 - 227467	N	N	N	N	N	N	N	DUP	DUP	DEL	DUP
1341-L6221	LOC159015	500456 – 500479	N	N	N	N	N	N	N	DUP	DUP	DEL	DUP
1145-L0702	SHOXa exon 1	505166 – 505189	N	N	N	N	N	N	DUP	DUP	DUP	DEL	DUP
1146-L6220	SHOXa exon 2	511850 – 511873	N	N	N	N	N	N	DUP	DUP	DUP	DEL	DUP
1147-L0802	SHOXa exon 3	515405 – 515428	N	N	N	N	N	N	N	DUP	DUP	DEL	DUP
1148-L1331	SHOXa exon 4	521565 – 521588	N	DUP	N	N	DUP	DUP	N	N	DUP	DEL	DUP
1149-L0910	SHOXa exon 5	521748 – 521771	N	DUP	N	N	DUP	DUP	N	N	DUP	DEL	DUP
1150-L0911	SHOXa exon 6	525296 – 525319	N	DUP	N	N	DUP	DUP	N	N	DUP	DEL	DUP
1151-L0708	ATG	531744 – 531767	N	DUP	N	N	DUP	DUP	N	N	DUP	DEL	DUP
1152-L0709	STOP SHOXa	538116 – 538139	N	DUP	N	N	DUP	DUP	N	N	N	DEL	DUP
5642-L5096	Xp22.32-PAR	594959 – 594982	N	N	N	N	N	N	N	N	N	DEL	DUP
5643-L5097	Xp22.32-PAR	626171 – 626194	N	N	N	N	N	N	N	N	N	DEL	DUP
5644-L5098	Xp22.32PAR	686180 – 686203	N	N	N	N	N	N	N	N	N	DEL	DUP
5645-L5099	Xp22.32PAR	729273 – 729296	N	N	N	N	N	N	N	N	N	DEL	DUP
5646-L5100	Xp22.32PAR	737731 - 737754	N	N	N	N	N	N	N	N	N	DEL	DUP
5647-L5101	Xp22.32PAR	743187 - 743210	N	N	N	N	N	N	N	N	N	DEL	DUP

Fig. 1. MLPA results obtained with P018 *SHOX* probe mix (MRC Holland) on the Xp region in five patients with type I MRKH syndrome. A, Schematic representation of *SHOX* gene and its flanking regions. MLPA probes are positioned on the *SHOX* exons and 5′ UTR and its flanking regions. The bars below the physical map indicate the various sized duplications observed in the familial patient 1 (Family 2), sporadic patients 2 and 3, and the two affected sisters (II-3 and II-4) of Family 1. For Patients 2 and 3, the telomeric boundary of the duplication was not defined (dashed lines) because the region was not covered by the MLPA probes. Physical position refers to Genomic Bioinformatic Group of University of California Santa Cruz (UCSC) Genome Browser on Human Mar. 2006 Assembly (hg18). B, Detailed list of the probe composition of P018 *SHOX* mix: the precise position of each probe is indicated, and the results for all members of Family 1 (including the two MRHK syndrome-affected sisters) and the other three patients with MRKH syndrome are shown. The last two columns refer to the positive controls (carriers of sex chromosome aneuploidies) for the deletion and the duplication of the whole targeted region.

it was not possible to establish the telomeric boundary of the duplication in Patients 2 and 3.

Controls

Analysis of DNA from the 53 healthy female controls using the P018 *SHOX* kit revealed no *SHOX* gene duplications. In six controls, probe signal patterns of >1.3 beyond the *SHOX* gene (>400 kb distance) were found for the following probes: 5650-L5104 (*SHOX* gene downstream and two controls), 5651-L5105 (*SHOX* gene downstream and two controls), 1170-L0710 (*CSF2RA* and one control), and 1153-L0712 (*ASMT* and one control). Analysis using the Database of Genomic Variants (available at: http://projects.tcag.ca/humandup/) indicated that the observed rearrangements were common polymorphisms.

Inheritance pattern of the *SHOX* duplication in Family 1

In Family 1, genotyping was performed using polymorphic markers in the PAR1 to trace the father-to-daughter inheritance of the region containing the *SHOX* gene duplication.

The results are shown in Figure 2B. The two affected sisters and their father shared the same haplotype of the PAR1, including *SHOX* gene. The centromeric boundary of the shared region was marked by SNP rs28631450, which is located approximately 1 kb downstream of *SHOX* gene, as shown in Figure 2A.

To confirm the intragenic *SHOX* gene duplication detected in Patients II-3 and II-4, quantitative genotyping of the heterozygous intragenic *SHOX* SNPs was performed using pyrosequencing. This demonstrated that the paternal rs3748527 (intron 5)

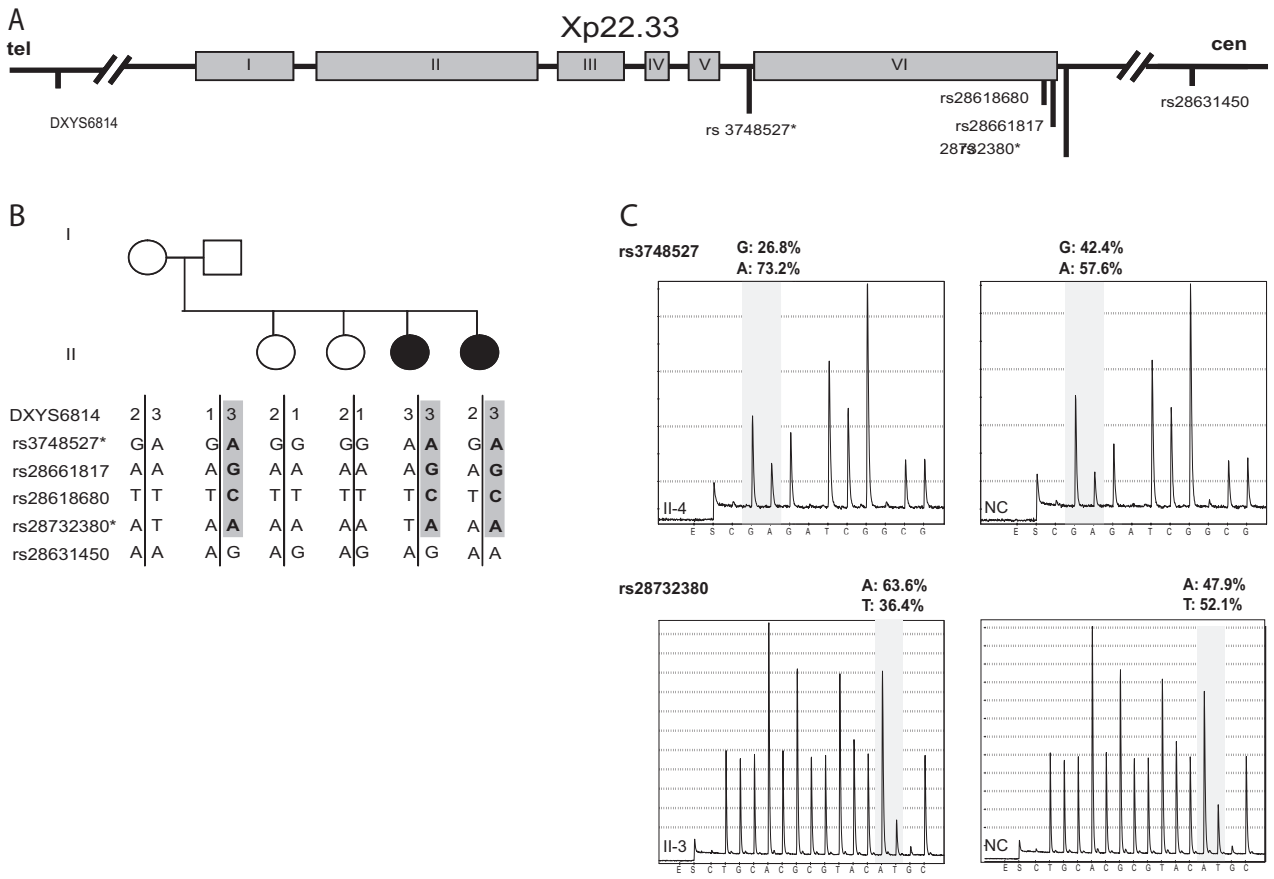


Fig. 2. Analysis of the *SHOX* region in Family 1. A, Representation of the *SHOX* genomic region. The positions of the polymorphisms used for the pyrosequencing (*) and haplotyping analyses are indicated. B, Segregation analysis using polymorphic markers encompassing the *SHOX* and flanking regions: the affected sisters (II-3 and II-4), but not the healthy sisters, inherited the haplotype containing the duplicated *SHOX* region from their father (shaded). C, Pyrograms of heterozygous SNPs located within *SHOX* gene of Patients II-3 and II-4 and one control (NC). The percentage of each allele contribution is indicated. Patients II-3 and II-4 show imbalances in the allele ratios, consistent with the presence of the duplication.

and rs28732380 (intron 6) alleles were present at a twofold dosage compared with the maternal alleles (Fig. 2C). Conversely, the contribution of the maternal and paternal alleles of the rs28475531, rs28574910, rs28433123, rs1894339, and rs2238842 loci, which are beyond and telomeric to the duplication, was balanced (data not shown).

The analysis was repeated in 10 normal control samples, which showed a balanced allele contribution for all heterozygous loci (data not shown).

DISCUSSION

The genetic basis of MRKH syndrome has been extensively investigated using a variety of approaches. These include (i) mutational analysis of candidate genes[10,13,18–22]; (ii) conventional cytogenetics,[5–12] fluorescent in situ hybridization analysis[1]; and (iii) investigation of whole genome imbalances by array-CGH.[15,16] *WNT4* mutations have been reported only in four patients all displaying hyperandrogenism, although most of the patients with this feature remain with an unknown etiology.[23–25] The lack of *WNT4* mutations in all the 12 patients with hyperandrogenism belonging to the cohort here tested is consistent with these findings.

Although familial aggregations are a valuable resource for the identification of disease genes, no previous study of MRKH syndrome families has identified any underlying genetic lesion.[13] This study used a two-step MLPA strategy (subtelomere screening and *SHOX* gene analysis) in a two-generation family with two MRKH type 1 syndrome sisters and healthy relatives (Family 1) to determine whether any subtelomeric imbalance cosegregated with the MRKH syndrome phenotype. A duplication of *SHOX* gene was detected and the involved genomic region (encompassing exons 4, 5, and a portion of exon 6) defined. This imbalance was shared by both sisters with MRKH syndrome and their healthy father but was lacking in the mother and the two unaffected sisters. In Family 1, the father showed no feature of MRKH syndrome (genital, renal, auricular, or skeletal anomalies).[30,31] It is, therefore, plausible that this imbalance is not associated with any clinically significant anomaly in the transmitting males. Conception of the two MRKH syndrome-affected daughters involved paternal gametes carrying the imbalance, whereas conception of the two nonaffected sis-

ters involved normal paternal gametes. This observation is consistent with the Shokeir's[14] hypothesis arguing for a dominant transmission of MRKH syndrome from unaffected male carriers. The report of two infertile paternal aunts in the family described in this study is intriguing. Although it was not possible to assign a definite diagnosis of MRKH syndrome in these women, our family provides some support to the paternal origin of MRKH syndrome in some pedigrees.

Interestingly, application of the MLPA approach in 28 additional women with MRKH syndrome led to the identification of *SHOX* gene duplications in three unrelated patients. One of these patients was a familial case from Family 2 (the sister was affected by MRKH type I syndrome), whereas the other two patients were sporadic. These five patients heterozygous for *SHOX* gene duplications had a clinical diagnosis of MRKH type I syndrome. On the whole, of 15 patients with MRKH type I syndrome in this cohort, one third displayed *SHOX* gene duplications. This suggests that partial *SHOX* gene duplications are implicated in both familial and sporadic forms of MRKH type I syndrome. *SHOX* gene is located within the PAR1 (Xp22.33; Yp11.3) and displays an "autosomal" rather than a sex-linked inheritance. In common with other genes in this region, *SHOX* escapes X inactivation. In addition, *SHOX* is the only known disease gene within PAR1, and its loss of function mutations (point mutations or deletions) being causal factors of disorders of bone development[26] including Leri-Weill dyschondrosteosis, Langer mesomelic dysplasia, and different forms of short stature. Three *SHOX* gene copies are found in some sex chromosome aneuplodies, which may be associated with tall stature in the absence of any distinct skeletal anomaly.[26] *SHOX* gene is a member of the paired-related *HOX* family, which is highly conserved among species. However, in contrast to other *HOX* family genes, *SHOX* is absent in the mouse and in rodent species.[32] *HOX* genes have been considered candidates for MRKH syndrome being involved in the development of the müllerian ducts.[33,34] However, mutations of *HOX* genes in mouse do not result in a MRKH-like phenotype.[35] This may be attributable to the absence of *SHOX* homologues in rodents.

SHOX gene encodes two differentially spliced mRNAs, SHOXA, and SHOXB. Its expression has been demonstrated in fetal and prepubertal growth plate chondrocytes. This is consistent with a role in bone development and final height.[36] SHOXB is expressed in fetal kidney tissue, which is closely involved in the development of the urogenital system.[26,36] Although there have been frequent reports of deletions within the *SHOX* gene region,[37–43] *SHOX* duplications are apparently very rare, only six cases having been described.[39,44–46] In these cases, the duplications included the entire *SHOX* gene coding region with different extragenic boundaries. No causal relationship between duplication of *SHOX* gene and any distinct phenotype has yet been established. Tachdjian et al.[45] described a *SHOX* gene duplication in a woman with premature ovarian failure, which displayed a complex rearrangement including Xq21.31 deletion. Thomas et al.,[46] in describing a previously published patient[39] and three novel families, hypothesized that complete *SHOX* gene duplications are associated with idiopathic tall stature. However, Roos et al.[44] rejected the hypothesis of "susceptibility to tallness" while reporting a patient with *SHOX* gene duplication and normal growth.

It is unclear why *SHOX* duplications are rare in comparison with *SHOX* deletions, because duplications and deletions have a reciprocal relationship, and are the simultaneous products of nonallelic homologous recombination during meiosis. One hypothesis is that duplications may be subject to negative selection, because they induce infertility in women. It is also possible that duplications may be overlooked in the normal male carriers who have no affected female progeny. The rearrangements described in this study did not encompass the complete *SHOX* gene sequence but involved different *SHOX* exons. MLPA screening of 53 healthy women and consultation of the Database of Genomic Variants (available at: http://projects.tcag.ca/humandup/) excluded any coincidental association between müllerian abnormalities and partial *SHOX* duplications.

In this cohort, none of the five patients with MRKH syndrome with a *SHOX* gene duplication showed skeletal abnormalities, which are expected in individuals with *SHOX* gene haploinsufficiency, and height values were within the expected parental targets. Although the consequences of partial *SHOX* duplications at the protein level are unknown, it is likely that they result in the production of aberrant proteins. It may be hypothesized that a gain of function, interfering at a specific time point with early embryonic development, could be implicated in the development of MRKH syndrome rather than a loss of function resulting in absence of the gene product. This point should be investigated by targeted studies.

The data obtained in this study should encourage further investigation of MRKH type I syndrome and suggest the involvement of *SHOX* gene in müllerian structures development. Future investigations should define the significance of heterozygous *SHOX* duplications and elucidate the mechanisms underlying the dysruption of early müllerian structure development.

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