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Clinical and molecular characterization of Chilean patients with Léri-Weill dyschondrosteosis

Abstract

Aim: Léri-Weill dyschondrosteosis (LWD) is a mesomelic dysplasia with disproportionate short stature associated with short stature homeobox-containing gene (SHOX) haploinsufficiency. The objective of this study was to improve the diagnosis of patients with suspected LWD through molecular analysis.

Methods: Twelve patients from 11 families with a clinical diagnosis of LWD were analyzed with multiplex ligation-dependent probe amplification to detect deletions and duplications of SHOX and its enhancer regions. High resolution melting and sequencing was employed to screen for mutations in SHOX coding exons.

Results: The molecular-based screening strategy applied in these patients allowed detection of five SHOX deletions and two previously unreported SHOX missense mutations.

Conclusion: Molecular studies confirmed the clinical diagnosis of LWD in seven out of 12 patients, which provided support for therapeutic decisions and improved genetic counseling in their families.

Keywords: Léri-Weill dyschondrosteosis; Madelung deformity; short stature; SHOX haploinsufficiency.

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Introduction

Léri-Weill dyschondrosteosis (LWD; MIM 127300) is a mesomelic dysplasia with disproportionate short stature described by Léri and Weill in 1929 (1). Its pathognomonic sign is a wrist deformity visible clinically or by X-ray that corresponds to an anterior subluxation of the radius and cubitus, known as the Madelung deformity (2). Other less specific clinical characteristics of LWD are short and bowed forearms and lower legs, short-metacarpal and metatarsal bones, high arched palate, short neck and muscular hypertrophy (3, 4).

Complete or partial short stature homeobox-containing gene (*SHOX*; MIM 312865) deletions as well as deletions of enhancer regions are the most common etiology of patients with LWD (5–9). *SHOX* missense mutations or duplications are detected to a lesser extent (5, 6, 10). *SHOX* is located in the pseudoautosomal region 1 (PAR1) on the short arm of both sex chromosomes (11). Genes in the PAR1 escape X inactivation, therefore, both copies of *SHOX* are expressed in men and women (12). *SHOX* codifies for a transcription factor with two characteristic domains, a homeobox domain responsible for specific DNA and protein binding (11), and an OAR domain involved in transactivation (13). Few transcriptional targets of SHOX have been described: *FGFR3* (14), *AGC1* (15), and *NPPB* (16); all are involved in skeletal development.

Other conditions associated with growth retardation, whose etiologies may be related to *SHOX* anomalies are Turner syndrome (17), Langer mesomelic dysplasia (MIM 249700) (4) and cases of idiopathic short stature (MIM 300582) (8, 11, 18, 19). It has been shown that patients with *SHOX* haploinsufficiency may benefit from growth hormone (GH) therapy (20–22). An early molecular diagnosis is essential for GH therapy as there is a time-sensitive window for treatment. Consequently, an efficient molecular diagnostic is of utmost importance to make a correct therapeutic decision.

The aim of this study was to perform a detailed molecular analysis of the PAR1 and coding exons of *SHOX*, in order to confirm the clinical diagnosis of LWD in a group of Chilean patients.

Materials and methods

Subjects

Twelve children from 11 families, diagnosed with LWD were recruited at the Institute of Maternal and Child Research, School of Medicine, University of Chile in Santiago, Chile. At least two of the following

criteria were fulfilled by the 12 recruited patients: height under –2 standard deviations (SDS) for age and gender (National Center for Health Statistics) (23); armspan/height ratio under 0.96, Madelung deformity and/or a family history suggestive of short stature and/or Madelung deformity in a first or second degree relative. This study, as well as informed consent for DNA extraction, was approved by the Ethics Committee of Hospital Clinico San Borja – Arriarán, Santiago, Chile.

Deletion and duplication analysis

The multiplex ligation-dependent probe amplification (MLPA) SHOX Kit (P018-E1) was employed to search for PAR1 deletions and duplications, using conditions specified by the manufacturer (MRC-Holland, Amsterdam, The Netherlands). This kit contains probes for each exon of *SHOX*, as well as probes upstream and downstream of *SHOX*, where *SHOX* regulatory elements are located. Furthermore, several probes in the X-specific region of the X chromosome were included to characterize large deletions. Finally, ten autosomal reference probes were included for normalization.

MLPA data was initially visualized with Peak Scanner Software v1.0 (Applied Biosystems, Foster City, CA, USA), and then the peak area data was imported to an Excel spread sheet (Microsoft, Redmond, WA, USA) for simple copy number calculations, as described previously (24). A value below 0.7 or above 1.3 was regarded as indicative of a heterozygous deletion (copy number change from two to one allele), or duplication (copy number change from two to three or more alleles), respectively.

SHOX point mutation screening

SHOX point mutations, small deletions and insertions were screened by high resolution melting (HRM) analysis. Briefly, the *SHOXa* coding exons were amplified as previously described (25). The PCR conditions were 1×HotShot™ Gold PCR Mastermix (Microozone, Southampton, UK); 0.3 μM each oligonucleotide; 1×LCGreen™ Plus + (Idaho Technology Inc., Salt Lake City, UT, USA); 5% DMSO and 15 ng genomic DNA in a total volume of 10 μL. Amplification products were analyzed in a 96-well Light-scanner™ HR96 system (Idaho Technology Inc., Salt Lake City, UT, USA) and those exons with abnormal profile relative to control samples (at least three) were subsequently sequenced on an ABI 3130XL (Applied Biosystems). At least three controls with known nucleotide changes for each amplicon were studied as controls.

Identified mutations were screened in relatives and healthy controls by restriction analysis. In brief, 5 μL of the PCR product obtained with primers flanking the mutation under study were incubated with appropriate restriction enzymes under conditions specified by the manufacturer (New England Biolabs Inc., Ipswich, MA, USA). The digested products were separated on a 2% agarose gel and stained with SYBR® Safe DNA Gel Stained (Invitrogen; Life Technologies, Carlsbad, CA, USA).

Results

Five *SHOX* deletions were detected by MLPA (42% of analyzed patients). Deletion sizes ranged from ~150 to 8545 kb, and included part or all *SHOX* genomic sequences (Figure 1). Three patients (LWS1, 2 and 16) may share the same deletion extensions, spanning ~963 kb from the p-telomere to probe L15508. Patient LWS19 exhibited a deletion that extended to probe L04577 (~6235 kb), located beyond the PAR1 boundary. Patient LWS12

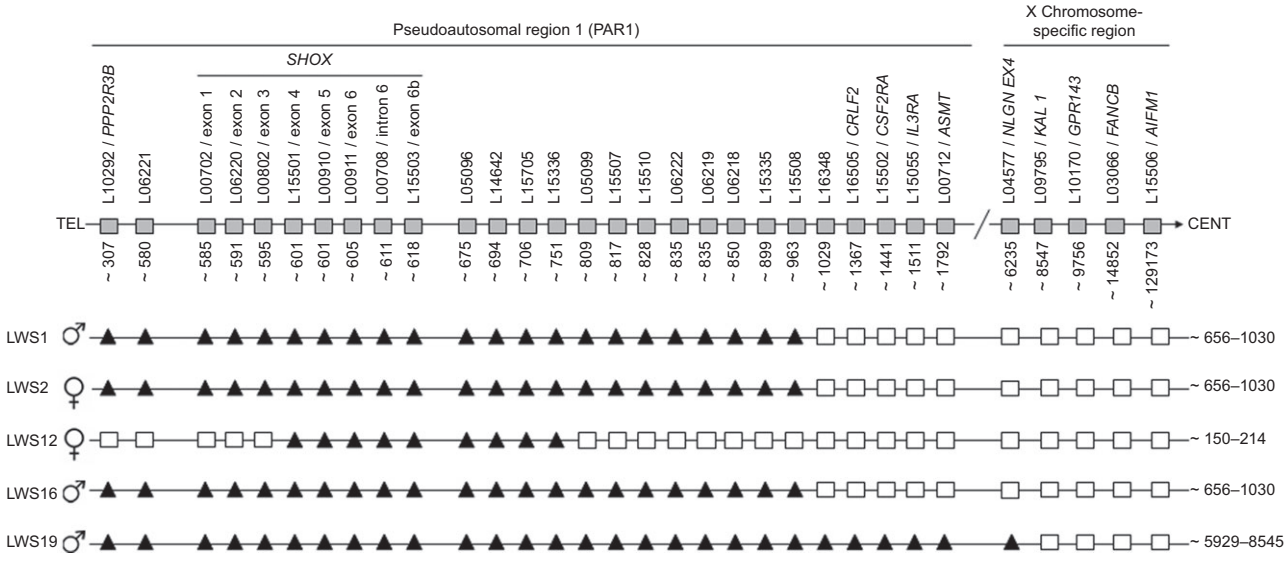

Figure 1 Pseudoautosomal region 1 (PAR1) deletions detected by multiplex ligation-dependent probe amplification (MLPA), in patients with Léri-Weill dyschondrosteosis (LWD). The name and position (kb from p-telomere) of each MLPA SALSA P018E-1 probe is indicated above the grey squares (diagram not drawn to scale). Genomic positions are according to the X chromosome sequence NC_000023.10, NCBI build 37.1. To the left, we depict the patient codes and gender. Empty squares indicate normal copy number whilst black triangles indicate the presence of a deletion. To the right, the deletion size is indicated.

Table 1 Clinical characteristics of Léri-Weill dyschondrosteosis (LWD) patients with short stature homeobox-containing gene (*SHOX*) anomalies.

LWD Patients							Affected parents
Patient code	Age (gender)	*SHOX* anomaly	Birth length (SDS)	Current height (SDS)	Armspan/height (ratio[a])	Madelung deformity (−/+)	Height SDS
LWS1	7 years 10 months (M)	*SHOX* deletion	−0.37	−2.96	0.93	−	−2.64 (F)
LWS2	7 years 2 months (F)	*SHOX* deletion	−0.12	−2.81	0.94	+	−3.66 (F)
LWS5	17 years 1 months (F)	c.439C>A (p.R147S)	+0.28	−2.65	0.95	+	−2.04 (M)
LWS12	9 years 8 months (F)	Partial *SHOX* deletion (ex4-6b)	−0.54	−3.07	0.92	+	−3.42 (M)
LWS16	10 years 4 months (M)	*SHOX* deletion	−0.75	−2.52	0.96	+	−3.42 (M)
LWS19	3 years 6 months (M)	*SHOX* deletion	−0.75	−1.53	0.96	−[b]	−3.57 (M)
LWS24	3 years 3 months (F)	c.778G>C (p.A260P)	−0.98	−2.46	0.99	−[b]	−3.91 (M)

SDS, standard deviations. [a]A value <0.96 suggests mesomelic shortening. [b]Children may develop MD later in life. (M) mother, (F) father.

harbored a partial *SHOX* deletion that extended from exon 4 to ~134–191 kb downstream of *SHOX*.

The mean height of the patients with PAR1 deletions was −2.57±0.55 SDS. All patients had an armspan/height ratio ≤0.96, which indicates mesomelic shortening, and three patients had the Madelung deformity (3/5). Deletions were transmitted by the father in two families and by the mother in three. The mean height of affected parents was −3.34±0.36 SDS (Table 1).

Seven patients, in whom no deletions were detected, were analyzed for point mutations, small deletions or insertions in the *SHOXa* coding exons and intron-exon boundaries. Two heterozygous missense substitutions were detected, c.439C>A (p.R147S) and c.778G>C (p.A260P), in exons 3 and 6a, in patients LWS5 and LWS24, respectively (Figure 2A–D). Both mutations are novel according to the Human SHOX Mutation Database (http://www.hd-lovd.uni-hd.de/) (26). The height of these patients was −2.65 SDS (LWS5) and −2.46 SDS (LWS24), respectively. An armspan/height ratio ≤0.96 and the Madelung deformity was only detected in the patient with the c.439C>A mutation (Table 1). Both mutations were transmitted by the mother (Figure 2E and F), their heights were −2.04 SDS (c.439C>A) and −3.91 SDS (c.778G>C), respectively. The missense mutation c.778G>C was not detected in 72 alleles from 36 healthy volunteers not related to the patients.

Discussion

PAR1 anomalies were detected in seven out of 12 Chilean patients with LWD. Cosegregation of the PAR1 alterations with the phenotype was observed in all families. Five of these alterations were *SHOX* deletions, four complete and one partial. The remaining two PAR1 alterations were heterozygous missense *SHOX* mutations, and neither have been previously described. Mutation c.439C>A (p.R147S) is the fourth mutation reported at the same codon in patients with LWD and ISS; p.R147H (27), p.R147P (28) and p.R147L (Esoterix, unpublished). Arginine 147 is a conserved amino acid located in the homeodomain of SHOX, which allows for specific binding to the palindromic DNA sequences 5′ -TAAT(N)ATTA- 3′ (13). The second mutation detected, c.778G>C, is predicted to result in the substitution of alanine 260 to proline (p.A260P). This mutation is located between the homeodomain (117–176) and the transactivation OAR domain (274–287) of SHOX. Even though there are no functional studies for mutations located in this region, predictive analysis of the p.A260P mutation using Alamut® Mutation Interpretation Software (Interactive Biosoftware, Rouen, France) indicates that it affects a moderately conserved amino acid residue and may be deleterious. Moreover, this mutation has not been observed in more than 2500 Caucasian controls (NHLBI Exome Sequencing Project, http://evs.gs.washington.edu/EVS/), or in 36 healthy Chilean volunteers, which suggests that it is not a normal variant in the Chilean population.

Mutation detection rate in this study was 58%, with a distribution similar to other studies, where PAR1 deletions account for approximately 80%, and point mutations account for 20%, of all LWD anomalies detected (29). Unexpectedly, among PAR1 deletions detected, no downstream *SHOX* deletions were observed, which differs from results of European studies where downstream deletions have been observed in 15%–45% of

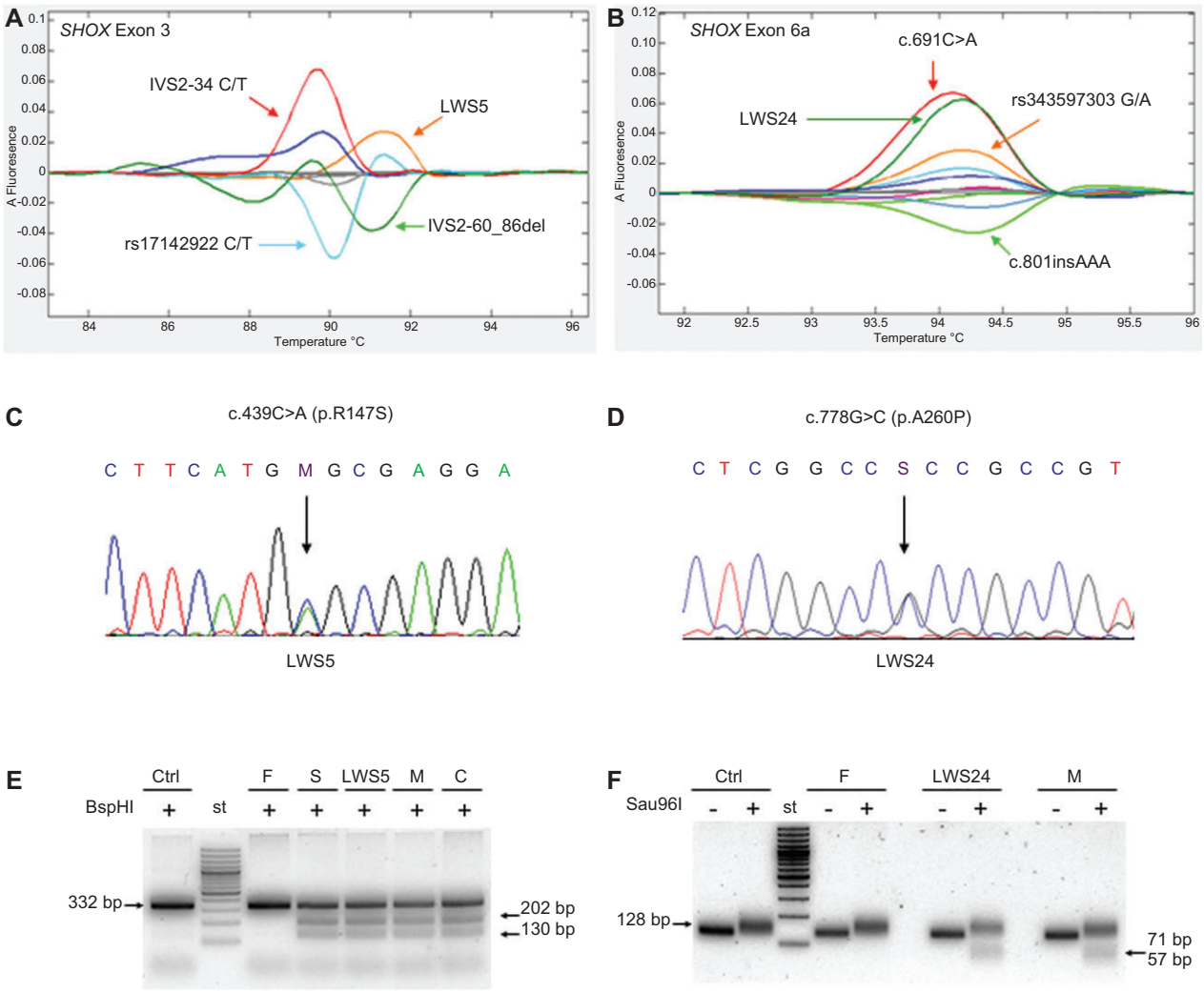

Figure 2 *SHOX* missense mutations detected in patients with Léri-Weill dyschondrosteosis (LWD). Results of the high resolution melting (HRM) analysis for *SHOX* exon 3 (A) and 6a (B) of patients (LWS5 and LWS24, respectively) and controls with known nucleotide changes are indicated with arrows. Sequence analyses of *SHOX* exon 3 (C) and 6a (D) show a heterozygous substitution of cytosine c.439 by adenine (M) in patient LWS5 and a substitution of guanine c.778 by cytosine (S) in patient LWS24 (indicated by arrows). (E) BspHI endonuclease restriction analysis for c.439C>A showed three fragments (332, 202 and 130 bp) in patient LWS5, her mother (M), her sibling (S) and a first cousin (C). The two lower fragments result from mutant amplicon (332 bp) digestion at positions 202–206 (fragments 202 and 130 bp) where the c.439C>A mutation generates a BspHI site. (F) Substitution c.778G>C give rise to a Sau96I restriction site at positions 71–74 of the 128bp amplicon, which produces two fragments (71 and 57 bp), not separated on the agarose gel. This band was detected in the patient LWS24 and her mother (M), but not in her father (F) or the control (C). St, 100 bp molecular weight standard.

LWD patients (7–9, 30). The only complete molecular characterization of South American patients with LWD published up to now includes eight Brazilian cases that did not have any downstream deletions (31). As most South American populations share an Amerindian origin, it is possible that the lack of these deletions has an ethnic explanation. Recruitment and analysis of additional LWD patients from our country will answer this question.

In summary, molecular characterization of a cohort of Chilean LWD patients resulted in: i) the detection of pathogenic PAR1 deletions in five out of 12 (42%) patients; and ii) the identification of two novel *SHOX* substitutions that have not been previously described: c.439C>A (p.R147S) and c.778G>C (p.A260P). This molecular-based screening strategy allows confirmation of the clinical diagnosis of LWD, helping to implement appropriate therapy and provide genetic counseling for the families of patients with these skeletal dysplasias.

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