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A Deletion of More than 800 kb Is the Most Recurrent Mutation in Chilean Patients with *SHOX* Gene Defects

Helena Poggi[a] Alejandra Vera[a] Carolina Avalos[b] Marcela Lagos[a] Cecilia Mellado[c, f] Mariana Aracena[c] Teresa Aravena[f, g] Hernan Garcia[b] Claudia Godoy[b] Andreina Cattani[b] Loreto Reyes[b] Patricia Lacourt[e] Hana Rumie[e] Veronica Mericq[d] Marta Arriaza[h] Alejandro Martinez-Aguayo[b]

[a]Molecular Biology Laboratory, Clinical Laboratory Department, [b]Endocrinology Unit and [c]Genetics Unit, Division of Paediatrics, and [d]Institute of Maternal and Child Research, Faculty of Medicine, Pontificia Universidad Católica de Chile, [e]Endocrinology Paediatrics Unit and [f]Genetics Unit, Complejo Asistencial Dr. Sotero del Rio, and [g]Genetics Unit, Hospital Clínico de la Universidad de Chile, Santiago, and [h]Endocrinology Paediatrics Unit, Hospital Dr. Gustavo Fricke, Viña del Mar, Chile

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SHOX deficiency · Short stature · Leri-Weill syndrome · Langer mesomelic dysplasia

Abstract
Background: Deletions in the *SHOX* gene are the most frequent genetic cause of Leri-Weill syndrome and Langer mesomelic dysplasia, which are also present in idiopathic short stature. ***Aim:*** To describe the molecular and clinical findings observed in 23 of 45 non-consanguineous Chilean patients with different phenotypes related to *SHOX* deficiency. ***Methods:*** Multiplex ligation-dependent probe amplification was used to detect the deletions; the *SHOX* coding region and deletion-flanking areas were sequenced to identify point mutations and single-nucleotide polymorphisms (SNPs). ***Results:*** The main genetic defects identified in 21 patients consisted of deletions; one of them, a large deletion of >800 kb, was found in 8 patients. Also, a smaller deletion of >350 kb was observed in 4 patients. Although we could not precisely determine the deletion breakpoint, we were able to identify a common haplotype in 7 of the 8 patients with the larger deletion based on 22 informative SNPs. ***Conclusion:*** These results suggest that the large deletion-bearing allele has a common ancestor and was either introduced by European immigrants or had originated in our Amerindian population. This study allowed us to identify one recurrent deletion in Chilean patients; also, it contributed to expanding our knowledge about the genetic background of our population.

Introduction

The short stature homeobox-containing gene *(SHOX)* resides in the telomeric pseudoautosomal region 1 (PAR1) on the short arm of both sex chromosomes and escapes X inactivation [1]. *SHOX* mutations occur with an estimated incidence of approximately 1 in 1,000 newborns, making mutations in this gene one of the most common monogenetic cause of defects associated with short stature and skeletal deformities [2]. Heterozygous *SHOX*

H. Poggi and A. Vera contributed equally to this work.

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E-Mail karger@karger.com
www.karger.com/hrp

Prof. Alejandro Martinez-Aguayo, MD
Division of Paediatrics, Faculty of Medicine
Pontificia Universidad Católica de Chile
Lira 85, piso 5, Santiago 8330074 (Chile)
E-Mail alemarti@med.puc.cl

gene defects are present in 50–90% of patients with Leri-Weill dyschondrosteosis (LWD; MIM 127300), a skeletal dysplasia with disproportionate short stature, mesomelic limb shortening, and the characteristic Madelung deformity. Langer mesomelic dysplasia (LMD; MIM 249700) is a more severe clinical form, in which 75% of patients are homozygous or compound heterozygous for *SHOX* mutations. In addition, 2–15% of children with idiopathic short stature (ISS) have defects in the *SHOX* gene, and when cases are selected by disproportionate ISS, this frequency can be even higher (22%) [3]. Finally, almost 100% of girls with Turner syndrome have an affected phenotype due to X chromosome haploinsufficiency.

Approximately 80% of genetic lesions in the *SHOX* gene are deletions, making them the most frequent type of mutation, followed by point mutations and duplications. Deletions may include only the gene, its upstream or downstream regulatory sequences, or both the gene and its regulatory sequences.

Here, we describe the molecular findings in patients with a phenotype suggestive of *SHOX* gene defects and characterize the type of mutations present in Chilean patients.

Materials and Methods

We studied a total of 45 non-consanguineous individuals (33 females and 12 males) from Santiago de Chile and other regions of our country. The patients, whose ages ranged from 1 month to 41 years, were evaluated in the Paediatric Endocrinology and Genetics Unit at Pontificia Universidad Católica de Chile. The anthropometric data included the height SDS, span/height ratio, the upper/lower segment ratio, and the forearm length/upper arm length ratio. Patients with LWD (n = 27), ISS with or without disproportion (n = 18), and 1 patient with LMD and Turner syndrome (45,X[10]/46,X,del(X)(p11.4)[15]) were included. The study protocol was approved by the Ethical Committee of the Faculty of Medicine of the Pontificia Universidad Católica de Chile.

Deletions and duplications were analysed by multiplex ligation-dependent probe amplification (MLPA) using the commercial SALSA MLPA probemix P018-F1 (MRC Holland, Amsterdam, The Netherlands) and GeneMarker software (SoftGenetics, LLC, State College, Pa., USA). In patients with a negative MLPA result, the entire *SHOX* coding region was sequenced and compared to the reference sequence (GenBank accession number: NC_000023.11).

To more precisely determine the extent of the deletion, array-based comparative genomic hybridization was performed using the Agilent Microarray 4x180K platform (Agilent Technologies, Santa Clara, Calif., USA) and Agilent CytoGenomics software to analyse the data. Primer sets were designed to sequence the areas flanking the deletion in the patients and in the 10 control samples. A large-fragment polymerase chain reaction was used to amplify the fragment resulting from the deletion.

Results

Of the 45 subjects studied, 23 were found to have *SHOX* gene defects (16 females and 7 males); 21 had deletions, 3 had point mutations, and the patient with Turner syndrome and LMD had a deletion and a novel point mutation. In 8 patients, a deletion of >800 kb was found, extending from almost the end of the telomere (upstream of the *PPP2R3B* gene) to the downstream enhancer region covered by the MLPA probe 093335-L19679. Likewise, another 4 patients had deletions of >350 kb from the promoter region to *SHOX* gene intron 6 (online suppl. fig. 1; for all online suppl. material, see www.karger.com/doi/10.1159/000439109). The remaining 11 patients had individual deletions, ranging from 77 to >2,000 kb. A summary of the deletions found in this study is presented in figure 1.

Of the 3 point mutations detected, 2 have been described previously: p.Arg173Cys and p.Pro244Alafs*147. The new point mutation corresponded to the duplication of a cytosine at position 426 (c.426dupC; NM_000451.3) that causes a frameshift resulting in a premature stop codon (p.Asp143Argfs*39; online suppl. fig. 2). Thus, it is highly likely that this new mutation is deleterious for the protein and, therefore, pathogenic. This prediction is consistent with the patient's phenotype.

According to the array-based comparative genomic hybridization and MLPA results, the deletion breakpoints in the 8 patients with the common large deletion was located between 187113 and 192689 at the telomeric end and between 1019893 and 1033100 at the centromeric end (X chromosome, NCBI assembly GRCh36). When searching for primers to amplify the region encompassing the deletion breakpoint, only a few primers were obtained, due to the proximity of this end to the telomere. Therefore, we were unable to amplify a fragment of the expected size and to determine the exact breakpoint location.

From the data obtained by sequencing the deletion-flanking areas, we identified 22 informative SNPs at the centromeric end. As 1 patient was homozygous for all SNPs but one, we were able to infer the haplotype likely to be associated with this recurrent deletion. This haplotype was present in 7 of the 8 affected patients; in the remaining subject, differences in 4 consecutive SNPs were observed. In all 10 control subjects with normal height, this haplotype was absent.

We found no significant differences in the anthropometric data according to the deletion. The patients with the deletion of >800 kb (n = 8, 7 LWD, 1 ISS) had a me-

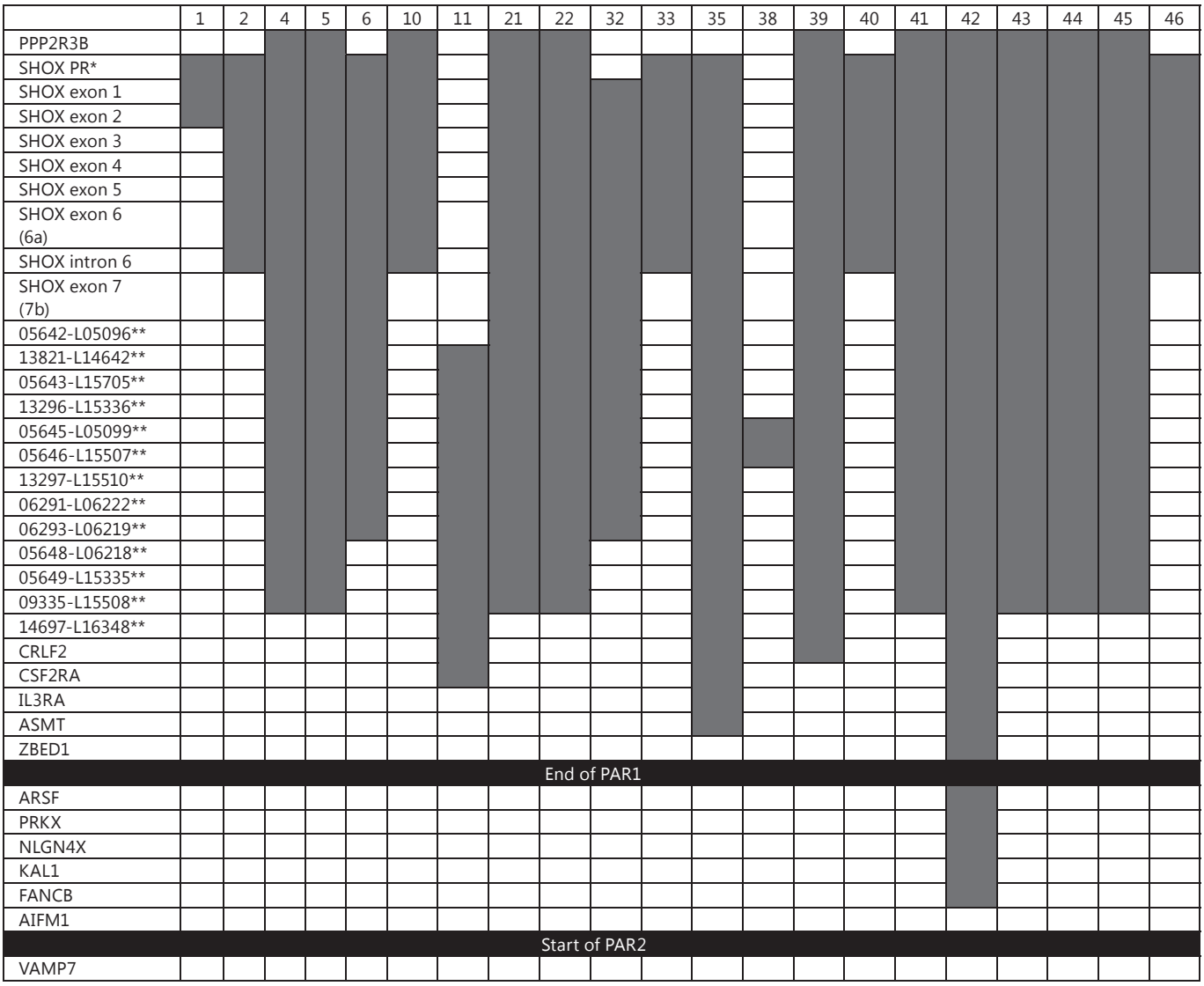

Fig. 1. Deletion extent according to the MLPA probes included in the SALSA P018-F1 kit. * LOC159015 (probe located 4.7 kb upstream of the *SHOX* gene; PR = promoter region). ** Probes in the *SHOX* gene enhancer region.

dian height SDS of –2.20 (min. –2.90; max. –1.51), and the LWD phenotype was identified in 6 fathers and 2 mothers with a median height SDS of –2.26 (min. –3.76; max. –1.70). While the patients with the shorter deletion of >350 kb (n = 4, 2 LWD, 2 ISS) showed a median height SDS of –2.57 (min. –2.83; max. –2.32). The patients with other genetic lesions (n = 10, 9 LWD, 1 ISS) had a median height SDS of –2.49 (min. –4.06; max. –0.87). The remaining data are reported in online supplementary table 1.

Discussion

In the present study, we describe molecular and clinical findings observed in 23 of 45 Chilean patients with different phenotypes related to *SHOX* deficiency, mainly LWD, but also ISS and LMD. The main genetic defects consisted of deletions; most identified deletions included the gene and its upstream and/or downstream regulatory regions, but 2 deletions only encompassed the downstream enhancer elements. This was expected, as deletions are the most frequent genetic lesions, and, also,

there are many reports on deletions affecting only downstream sequences [4]. Moreover, a recent report demonstrated that upstream deletions alone could also be responsible for the *SHOX* deficiency phenotype [5].

Among the *SHOX* deletions, one was found recurrently: a large deletion of >800 kb in 8 patients; however, it is important to note that another smaller deletion of >350 kb was observed in 4 patients. We conducted further studies to identify the breakpoint and exact size of the >800-kb deletion to determine if it was the same on all alleles. Although it was not possible to determine the deletion breakpoint due to the proximity of one end of the deletion to the telomere, a common haplotype was found in 7 of the 8 patients using informative SNPs. This result suggests that the deletion-bearing allele likely originated in a common ancestor, either in a European immigrant or in an Amerindian individual. The possibility of a common origin is also supported by the fact that this deletion has not been reported as recurrent in other populations. Interestingly (and further supporting our observations), the large >800-kb deletion recurrently found in this study was also observed in Chilean patients with LWD in a recent study performed by another group. Although the sample in that study was smaller, this deletion was present in 3 out of 7 patients with *SHOX* gene mutations [6]. To our knowledge, only 1 recurrent PAR1 deletion with identical breakpoints has been reported, although a common ancestor was excluded based on haplotype analysis [5]. The authors therefore attributed the recurrence of this deletion to multiple de novo events, in contrast to our observations where a common origin for the recurrent large deletion is the most likely explanation.

Regarding the relationship between the presence of a *SHOX* mutation (grouped into the >800-kb deletion, the >350-kb deletion, and other deletions and point mutations) and the patients' phenotype (clinical diagnosis), we did not find an association linking the clinical severity to the type of genetic lesion found. This finding was expected, as *SHOX* haploinsufficiency is known to have a high penetrance, with a highly variable clinical expression [1].

In summary, we identified one recurrent *SHOX* deletion in Chilean individuals with LWD and ISS and found evidence supporting a common origin for the >800-kb deletion. This study allowed us to characterize a sample of our population before implementing *SHOX* genetic analysis in the clinical setting, and it contributed to expand our knowledge about the genetic background of human disease-related genes in our population.

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Disclosure Statement

The authors declare no conflicts of interest.

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