CASE REPORT

Isolated haploinsufficiency of exon 1 of the *SHOX* gene in a patient with idiopathic short stature

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This paper reports the case of a 16-year-old woman with idiopathic short stature (ISS) who was detected to be haploinsufficient in only exon 1 of the short stature homeobox-containing (*SHOX*) gene by RQ-PCR and had two copies of the other six exons intact. The translation of the *SHOX* protein and of the *SHOX* promoter may be potentially affected if the deletion of exon 1 is extended further upstream. Further studies may help in determining the significance of partial exonic deletions of the *SHOX* gene in relation to ISS.

Idiopathic short stature (ISS) is essentially a failure to grow for unknown reasons and is a diagnosis of exclusion.^{1–3} It is generally agreed that, for a diagnosis of ISS, a child must be of normal size at birth, be proportionate in stature, have a normal growth hormonal response to stimulation tests and be more than 2 SD below the mean height for age.^{1–3} Currently, the aetiology for ISS has not been established. It is, however, believed that haploinsufficiency of the short stature homeobox-containing gene or the *SHOX* gene⁴ may account for ISS in more than 1–2% of all children with ISS.⁵ Other molecular defects such as missense and nonsense mutations, small deletions in the coding region and null alleles associated with intragenic *SHOX* deletion or its flanking markers can also account for ISS.^{6,7}

We report a patient with ISS who is haploinsufficient in only exon 1 of the *SHOX* gene and has two copies of the other six exons intact. A 16-year-old woman was referred for the assessment of short stature. She was born full term, with a normal birth weight of 2400 g, and has been short since she was a child. At 16 years, her height was 145.3 cm (on the third centile, –2.2 SD) and her weight was 53.5 kg (on the 75th centile). The adjusted mid-parental height was 152.9 cm, and her height was at the lower limit of her target range. She experienced menarche at 10 years and has had regular periods since.

Clinically, she was proportionate, with no dysmorphic features. She was pubertal, with Tanner stage 5 breast development. Her bone age was not delayed. Clinically, there seemed to be no possibility of growth hormone deficiency, as her height velocity, on the basis of previous records, was normal in her early years and was currently appropriate for her pubertal stage.

Genomic DNA was extracted from the leucocytes by the method recommended by QIAGEN. The QuantiTect SYBR Green PCR Kit (QIAGEN, Hilden, Germany) was used for carrying out the RQ-PCR. Standards consisting of varying amounts of normal DNA were set up for all the exons and the internal control gene *GAPDH*. Amplification was carried out in the Lightcycler (Roche, Penzberg, Germany) for DNA from the proband, as well as for 10 normal controls and two patients with Turner syndrome. A ratio was determined for

each exon, per individual, on the basis of the following formula:

$$\frac{(\text{number of } SHOX \text{ gene copies (patient)} / \text{number of } GAPDH \text{ gene copies (patient)})}{(\text{number of } SHOX \text{ gene copies (control)} / \text{number of } GAPDH \text{ gene copies (control)})}$$

The means and SD of the RQ-PCR ratios for each exon of the normal controls were calculated and the ratios of each exon obtained for the proband were compared with the lower limit of the respective exon to determine the deletion of one copy of the exon.

Table 1 gives the ratios determined for the proband and patients with Turner syndrome. Table 2 gives the ratios for the 10 normal controls. Ratios in the range of 0.2–0.5 were approximated to 0.5, indicating a deletion of one copy of the exon. Ratios in the range of 0.7–1.5 were approximated to 1.0, indicating the presence of two functional copies and hence no deletion.

Each exon of the proband's *SHOX* gene was also free from point mutation as observed after direct sequencing analysis (results not shown).

This is an interesting case of ISS in which only one copy of exon 1 is deleted, with two copies each of the other exons intact. Exon 1 makes up part of the 5' untranslated region of the *SHOX* transcript. As the 5' untranslated region has an important role in post-transcriptional regulatory pathways that control mRNA localisation, stability and translation efficiency,⁸ the absence of one copy of exon 1, which codes for this region, may potentially lead to the instability and rapid breakdown of the mRNA transcript, thus affecting the translation of the transcription factor.

Capping is an essential process, as it stabilises the mRNA by methylation,⁹ enhances the efficiency of splicing, protects the mRNA from premature degradation by nucleases and helps in effective transport of mRNA from the nucleus to the

Table 1 RQ-PCR ratios of the proband and two patients with Turner syndrome who acted as positive controls

Patient	Sex	Karyotype	Calculated ratio for exons							
			1	2	3	4	5	6a	6b	
Proband (ISS)	Female	46, XX	0.4	0.8	1.1	1.2	1.0	0.9	1.1	
TS1	Female	45, XO	0.4	0.5	0.5	0.5	0.5	0.5	0.5	
		46, X, i(X)	0.4	0.3	0.2	0.5	0.5	0.4	0.5	
TS2	Female	(qter→q10::q10→qter)								

ISS, idiopathic short stature, TS, Turner syndrome.

Abbreviations: ISS, idiopathic short stature; *SHOX*, short stature homeobox

Table 2 Statistical analysis of each exon of the normal controls

Normal controls	Exons						
	1	2	3	4	5	6a	6b
1	1.2	1.2	1.3	1.2	1.3	1.2	1.1
2	1.3	1.3	1.1	1.0	1.1	1.1	1.1
3	1.3	1.5	1.3	1.2	1.5	1.3	1.1
4	1.0	1.1	0.9	0.8	1.0	0.9	1.1
5	1.2	1.3	1.3	1.2	1.2	1.1	1.1
6	0.7	0.9	0.8	0.7	0.9	0.8	1.0
7	1.2	1.2	1.5	1.1	1.1	1.2	1.3
8	0.8	0.7	0.7	0.7	0.7	0.7	0.6
9	0.8	1.0	0.9	0.9	0.9	0.8	0.7
10	1.2	1.2	1.1	1.1	1.4	1.1	0.9
Mean	1.07	1.14	1.09	0.99	1.11	1.02	1.0
SD	0.23	0.23	0.26	0.20	0.25	0.20	0.21
Lower limit	0.63	0.69	0.58	0.59	0.63	0.62	0.59

Any value below the lower limit indicates a deletion of one copy of that exon. Lower limit = mean - 1.96 SD.

cytoplasm.¹⁰ The cap may not be formed properly by having only one copy of exon 1.

Conceptually, the potential consequence of the deletion of exon 1, which extends to include the upstream promoter, is haploinsufficiency of the *SHOX* gene, which would then explain the clinical phenotype of the proband, although we cannot ascertain the extent of the deletion on the basis of the microsatellite analysis of the patient's DNA.

To our knowledge, this is the first reported case of haploinsufficiency on only a single exon in ISS. Further studies may help in determining the significance of partial exonic deletions of the *SHOX* gene in relation to ISS.

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Take-home messages

- This is the first reported case of haploinsufficiency of a single exon of the *SHOX* gene in idiopathic short stature (ISS).
- The loss of one copy of exon 1 of the *SHOX* gene may potentially affect translation of the *SHOX* protein and the *SHOX* promoter if the deletion of exon 1 extends further upstream.
- This case opens the door to further studies on the significance of partial exonic deletions in relation to ISS.

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