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Pathogenic and likely pathogenic genetic alterations and polymorphisms in growth hormone gene (*GH1*) and growth hormone releasing hormone receptor gene (*GHRHR*) in a cohort of isolated growth hormone deficient (IGHD) children in Sri Lanka

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ABSTRACT

Objective: Genetic alterations in *GH1* and *GHRHR* genes are known to cause isolated growth hormone deficiency (IGHD). Of these, *GHRHR* codon 72 mutation has been reported to be highly prevalent in the Indian subcontinent, but among Sri Lankans its prevalence was low compared to reports from neighboring countries. The present study was therefore carried out to identify genetic alterations in the *GH1* gene and rest of the *GHRHR* gene in a cohort of Sri Lankan IGHD patients who tested negative for *GHRHR* codon 72 mutation.

Methods: Fifty five IGHD children negative for codon 72 (*GHRHR*) mutation were screened for gross *GH1* gene deletion by polymerase chain reaction (PCR) and restriction fragment length polymorphism technique. The coding, intronic and promoter regions of the *GH1* gene were sequenced in children who were negative for *GH1* deletion (N = 53). In a subset (N = 40), coding, flanking intronic and promoter regions of the *GHRHR* gene were screened by single strand conformation polymorphism/sequencing. Identified coding region and intronic variants were subjected to *in silico* analysis to ascertain pathogenicity. Family members available were screened for the significant variants observed in the index child.

Results: Gross *GH1* gene deletions, 6.7 kb and 7.0 kb were observed in one child each. One novel and 24 reported single nucleotide variants (SNVs) were observed in the *GH1* gene and its promoter. These included one reported pathogenic splice site mutation (c.172-2A > T) and one reported likely pathogenic missense mutation (c.406G > T). One large novel deletion of 5875 base pairs that included exon 1, one likely pathogenic novel SNV (c.211G > T) and 18 reported SNVs were observed in the *GHRHR* gene. Fourteen variants observed were of uncertain significance (8 in *GH1* and 6 in *GHRHR*), twenty three variants were likely benign (11 in *GH1* and 12 in *GHRHR*) and four variants were benign (4 in *GH1* and none in *GHRHR*).

Conclusion: In a cohort of IGHD children, six pathogenic or likely pathogenic genetic alterations of either *GH1* gene or *GHRHR* gene were found. These affected a total of six children. Pathogenic status of four of these had been reported in the literature. Novel SNV in the *GHRHR* gene was predicted to be pathogenic through *in silico* analysis. The large novel deletion is likely to be pathogenic as it included exon 1 of *GHRHR* gene. Analysis of other genes will be needed to ascertain the genetic cause of IGHD in the remaining children.

1. Introduction

The prevalence of short stature due to growth hormone deficiency (GHD) is reported to vary from 1/4000 to 1/10,000 in different populations. Most cases are sporadic with only 5–30% being familial [1–5]. *GH1* gene is located on the long arm of chromosome 17 (17q22-24) within the cluster of five paralogous genes (arranged in the order of 5′-*GH1-CSHP1-CSH1-GH2-CSH2*-3′) which are similar in sequences

Abbreviation: GH, Growth hormone; IGHD, Isolated growth hormone deficiency; SNV, Single nucleotide variant; TGP, Thousand genome project; ExAC, Exome aggregation consortium; MAF, Minor allele frequency/minor allele count

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[6,7]. It has five exons and four introns [8]. Regulation of *GH1* gene expression is mediated by proximal promoter and locus control regions. The proximal promoter region is reported to be highly polymorphic [9]. Growth hormone releasing hormone (GHRH) stimulates growth hormone synthesis and secretion [10] through GHRH receptor (GHRHR), a member of the G protein coupled receptor family located on the surface of somatotrophs [11]. *GHRHR* gene located on the short arm of chromosome 7 (7P14) [12] consists of thirteen exons [13]. It is now well recognized that *GH1* mutations are seen in IGHD type 1A, 1B and 2 whereas *GHRHR* mutations are seen in IGHD type 1B [14]. However there are hardly any reports of *GHRH* mutations leading to IGHD [15]. Several studies have reported that *GHRHR* codon 72 mutation is the commonest genetic alteration in IGHD in the Indian subcontinent [12,16,17]. However, we found this mutation to be less common in Sri Lankan IGHD patients compared to the prevalence reported from India and Pakistan [12,17,18]. In the present study we further screened a cohort of IGHD patients who were negative for *GHRHR* codon 72 mutation for possible genetic alterations in the *GH1* gene and its promoter as well as in the rest of the exonic regions of the *GHRHR* gene, flanking intronic regions and its promoter.

2. Materials and methods

Ethical approval for the study was obtained from ethics review committee of the Faculty of Medicine, University of Colombo. Informed consent was obtained from parents of all IGHD children and from adults with normal height recruited for the study before collecting clinical data and blood samples.

2.1. Subjects

Total of 55 IGHD children who were negative for codon 72 mutation of the *GHRHR* gene [18] were recruited for the study from Lady Ridgeway Hospital, Colombo, Sri Lanka. Characteristics of the IGHD cohorts screened for *GH1* and *GHRHR* are given in Table 1. They were confirmed to have growth hormone deficiency biochemically with no or suboptimal response to glucagon stimulation test. The recruited children were from different families except two children who were second degree relatives. Although they represented all nine provinces of the island, 54.5% were from the Western Province. They were diagnosed with IGHD between 2 years and 17 years of age. Nine children who were initially diagnosed as IGHD later developed deficiency of TSH or ACTH. Peripheral venous blood samples (2.5 mL) were collected from the recruited patients. Genomic DNA was extracted from isolated leukocytes using the salting out method [19].

2.2. Molecular genetic studies

All the patients were screened for 6.7 kb, 7.0 kb and 7.6 kb gross gene deletions of the *GH1* gene. *GH1* gene and its promoter were sequenced in those who were negative for gross gene deletions. Highly polymorphic segment of the promoter, untranslated region of exon 1 and intron 4 of *GH1* gene (where polymorphisms were observed in the affected children) were screened in 20 healthy adults with normal height. A subset of 40 IGHD children was screened for exonic and flanking intronic regions of the *GHRHR* gene and its promoter.

2.2.1. Identification of gross GH1 gene deletions

Gross gene deletions were identified using polymerase chain reaction/restriction fragment length polymorphism (PCR-RFLP) [20–22]. Specific segments of upstream and downstream homologous regions flanking *GH1* gene were PCR amplified with a single pair of primers [22]. Final PCR reaction mixture of 25 μL consisted of 250 ng of DNA, 1 × Green GoTaq® reaction Buffer, 3 mM $MgCl_2$, 200 μM deoxynucleotide triphosphate (Promega Corporation, Madison, WI, USA), 0.2 μM each forward and reverse primers (IDT Integrated DNA Technologies, Coralville, Iowa, USA) and 1.25 U of GoTaq® Flexi DNA polymerase (Promega). PCR conditions were: 94 °C for 6 min, 30 cycles of 94 °C for 1 min, 63 °C for 45 s and 72 °C for 3 min and final extension at 72 °C for 10 min. PCR products were purified with Wizard® SV Gel and PCR Clean-Up System (Promega) and digested with *Sma*I restriction endonuclease (New England Biolabs). The digestion reaction mixture (20 μL) consisted of purified PCR ampicons (5 μL), NE Buffer 4 (2 μL from 10 × concentration), *Sma*I restriction enzyme (20 U) and distilled autoclaved water (12 μL). Reactions were carried out overnight at 37 °C. Digested samples (5 μL) were subjected to electrophoresis in 10% polyacrylamide gel and stained with silver nitrate to visualize bands. DNA from a healthy adult with normal height was used as the control, and banding patterns compared to detect gross gene deletions.

2.2.2. Identification of genetic variants in the GH1 gene and its promoter region

Three pairs of overlapping primers were designed to cover the *GH1* gene and two forward primers and a reverse primer were designed to amplify the promoter region using nested PCR technique (Supplementary Table 1). The primer designed for the promoter region

Table 1
Baseline characteristics of the IGHD cohort screened for *GH1* gene and *GHRHR* gene.

		Phenotype of the cohort screened for *GH1* gene variants		Phenotype of the cohort screened for *GHRHR* gene variants	
		IGHD	Partial IGHD	IGHD	Partial IGHD
Number		31	24	24	16
Males (no)		21	18	18	10
Height SDS (range)		−7.3 to −1.95	−7.81 to −2.1	−6.63 to −1.95	−5.47 to −2.63
Age at diagnosis (range)		3Y 10M to 16Y 3M	2Y 2M to 14Y 3M	3Y 10M to 16Y 3M	3Y 5M to 14Y 3M
Family history of short stature present (No.)		3	3	2	3
Consanguinity of parents (No.)		5	2	2	1
Ethnicity	Sinhala	27	19	20	12
	Tamil	0	4	0	3
	Moor	4	0	4	0
	Mixed parentage	0	1[a]	0	1[a]
Pituitary imaging (MRI/CT)	Normal	16	17	12	12
	Hypoplastic pituitary	7	3	6	1
	Posterior pituitary affected	2	0	2	0
	Hypoplastic and posterior pituitary affected	4	3	4	3
	Not done	2	1	0	0

[a] Mixed parentage of Sinhala and Moor.