Growth hormone releasing hormone receptor codon 72 mutation in a cohort of Sri Lankan patients with growth hormone deficiency

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(Index words: short stature, growth hormone deficiency, growth hormone releasing hormone receptor codon 72 mutation)

Abstract

Introduction Growth hormone releasing hormone receptor *(GHRH-R)* codon 72 mutation is recognised as a common genetic cause of growth hormone deficiency (GHD) in the Indian subcontinent resulting in a characteristic lean phenotype. Genetic studies have not been previously carried out in Sri Lankans with GHD.

Methods Patients with GHD presenting to a tertiary care referral centre were studied for *GHRH-R* codon 72 mutation by PCR amplification and sequencing. The phenotype of the cohort was described as the BMI SDS (Body mass index standard deviation score) based on the anthropometric data at the time of diagnosis.

Results Among 91 patients from 88 families studied, eight (6 boys) carried the codon 72 mutation. The presence of this mutation was low among the Sinhalese ethnicity (3 out of 68) than among Tamil and Moor ethnicities. BMI SDS of <-2 was seen in 71% of mutation positive and 45.8% of mutation negative patients.

Conclusions Prevalence of *GHRH-R* codon 72 mutation in this group of GH deficient patients was 8.8%. The lean phenotype observed in 71% of the mutation positive patients was not a significant association when compared to a similar phenotype in 45.8% of the mutation negative patients.

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Introduction

Growth hormone (GH) is under stimulatory and inhibitory control by hypothalamic hormones, growth hormone releasing hormone (GHRH) and somatostatin respectively. GHRH acts on GHRH receptor (GHRH-R) located in the plasma membrane of anterior pituitary somatotrophic cells and signals the somatroph to synthesise and secrete GH. Thus GH deficiency (GHD) can result from genetic defects in *GH1* gene which codes for pituitary GH and *GHRH-R* gene coding for the GHRH receptor [1]. GHRH-R mutations are reported to be the cause of approximately 10% of isolated growth hormone deficiency with an autosomal recessive inheritance [2]. Genetic defects of *GHRH* and somatostatin are rare [3]. Mutations in genes downstream of *GH-1* comprise GH insensitivity syndrome [4].

GHRH-R mutation associated with isolated GHD (IGHD) was first described in two children in a consanguineous Indian Moor family [5]. This nonsense mutation (c.214 G<T, p.E72X) introduces a stop codon at codon 72 due to G to T transversion, which converts the amino acid coding codon, GAG to stop codon, TAG. The same mutation was demonstrated in two migrant Tamil brothers from Delft Island belonging to Sri Lanka and in a large consanguineous kindred from Sindh province in Pakistan [6, 7]. This mutation associated with a lean phenotype has a prevalence rate of 71% among patients with IGHD in Western India [8]. All patients of this study were either Hindus or from an inbred Moor community. In a large series of growth hormone deficient patients of multiple ethnicities, only 3.7% carried *GHRH-R* mutations [9]. Among them codon 72 mutation was the commonest being present in eight patients from two Asian and one Somalian pedigree in homozygosity and in two patients from one Asian pedigree in compound heterozygosity.

The *GHRH-R* codon 72 mutation has been reported as the commonest mutation among Asians with a majority

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having a lean phenotype [8]. We had observed that the phenotype of our patients with GHD was unlikely to be the commonly accepted phenotype of this condition. Molecular genetic studies on GHD have not been carried out previously in Sri Lanka and thus the presence of *GHRH-R* codon 72 mutation has not been demonstrated. The present study was done to screen a group of biochemically confirmed GH deficient children for the *GHRH-R* codon 72 mutation and to describe their phenotype in relation to the BMI SDS (Body mass index standard deviation score).

Methods

Ninety one patients with GHD confirmed by a single provocation test using glucagon followed up in the University Unit at the Lady Ridgeway Hospital, Colombo, were invited to participate in the study [10,11]. Written, informed consent was obtained from the parents and the patient when appropriate. Ethical Review Committee of the Faculty of Medicine, University of Colombo, approved the study.

Anthropometric data were recorded at the time of the study and clinical and biochemical data at the time of diagnosis were obtained from the patients' records. The anthropometric data were analysed as the standard deviation scores (SDS) calculated for BMI, height for age and weight for age using the LMS growth programme version 2.69 (2010) using World Health Organisation child and 5-19 growth reference standards (2006/2007). A lean phenotype was defined as a BMI SDS <-2.

Two to five ml of peripheral venous blood was obtained under aseptic conditions and stored at -20^{o}C until DNA was extracted using Wizard genomic extraction kit (Promega, USA). Polymerase chain reaction with specific primers (GACACCCAAATGGCTTGGCTCAT; reverse: GCCACTTCCAGATGAAAGCACCTC) was performed to amplify the region containing the codon 72 mutation. PCR reaction mixture contained 25 ng of DNA, 0.4 μM of each primer, 0.2 mM dNTP (Promega, Madison, WI, USA), 3.0 mM $MgCl_2$ and 1.25 units of Taq polymerase (Promega). PCR reaction of 40 cycles comprised of initial denaturation at 94°C for 4 minutes; denaturation, annealing and elongation at 94°C, 60°C and 72°C respectively for 30 seconds each; final elongation at 72°C for 10 minutes. PCR products were purified using Illustra-GFX column purification kit as per manufacturer's instructions (GE Healthcare Bio-Sciences Corp., Piscataway, NJ, USA). PCR fragment was subjected to direct sequencing using DYEnamic ET Dye Terminator Cycle Sequencing reagents on a MegaBACE 1000 automated DNA sequencer (GE Healthcare Bio-Sciences Corp). Resulting nucleotide sequences were subjected to bioinformatic analysis to identify possible mutations in the sequenced DNA fragment.

Results

All 91 patients (64 males) who were invited to participate gave consent for the study. They were from 88 families with 68 Sinhalese, 9 Tamil and 13 Moor patients and one child was the offspring of a Sinhalese mother and Moor father. Age distribution of the patients at the time of study is given in Table 1. Twenty two patients (24%) had biochemical evidence of deficiency of other anterior pituitary hormones at the time of diagnosis. Nine and three patients were deficient in TSH and ACTH respectively and 10 had combined TSH and ACTH deficiency. Gonadotrophin and prolactin levels were not assayed.

Eight (6 boys) of the 91 patients (8.8%) carried the codon 72 mutation in the *GHRH-R* gene. All eight patients had isolated GHD (IGHD) at the time of study. There were three Sinhalese patients, two Tamil brothers and three Moor patients, two of whom were siblings. Their ages at the time of study ranged from 3 years to 19 years and 6 months with a mean (SD) of 10.695 (5.987) years. Table 2 gives the distribution of the total sample and the mutation positive patients in the different provinces.

Fourteen children (of 13 families) were born to consanguineous parents. Five of them had the mutation, two of Sinhalese parents, two Tamil brothers and one Moor child. Of the other nine patients without the mutation, four had Sinhalese parents, two had Tamil and three had Moor parents. One child with the mutation was adopted by Sinhalese parents. A family history of GHD was seen in six patients from three families: a brother and sister each

Table 1. Age distribution of the study sample (n=91)

Age groups (years)	≤5	5 - ≤10	10 - ≤15	15 - ≤20	>20
Number of patients at the time of study	10	25	39	15	02

Table 2. Distribution of the study sample and the mutation positive patients in the different provinces

Province	*Number of patients (n=91)*	*Mutation positive (n=8)*
Western	42	0
Southern	4	0
Eastern	1	0
Northern	4	2
North Central	3	0
Central	14	3
North Western	11	2
Sabaragamuwa	8	1
Uva	4	0

of Sinhalese and Moor ethnicity born to non consanguineous parents and two Tamil brothers of a consanguineous union.

Five of the seven mutation positive patients (71%) in whom the anthropometric data were available at the time of diagnosis, had a BMI SDS <-2, and 38 (45.8%) mutation negative patients had a similar BMI SDS. This difference was not statistically significant (t=2.04, df=1, p>0.05).

Table 3. Anthropometric data of the patients at the time of diagnosis (n=90*)

	Mutation positive (7) n (%)*	*Mutation negative (83) n (%)*
BMI SDS		
<-2 (wasted)	5 (71.4)	38 (45.8)
-2 to +1 (normal)	2 (28.6)	40 (48.2)
+1 to +2 (over weight)	0	5 (6)
Height for age SDS		
<-3 (severe stunting)	7 (100)	72 (86.8)
<-2 to -3 (moderate stunting)	0	8 (9.6)
-2 to +2 (normal)	0	3 (3.6)
Weight for age SDS		
<-3 (severe wasting)	7 (100)	65 (78.3)
<-2 to -3 (moderate wasting)	0	9 (10.8)
-2 to +2 (normal)	0	9 (10.8)

*Anthropometric data of the mutation positive patients at the time of diagnosis was available for seven patients only

Discussion

Sri Lanka has a population comprising of several ethnic groups with majority being Sinhalese (74.9%). Tamil (11.2%) and Moor (9.2%) ethnic groups are the second and third commonest [12]. This is the first report of *GHRH-R* codon 72 mutation among native Sri Lankans. It was seen in homozygosity in 8.8% of our study sample. This mutation is reported to show an autosomal recessive inheritance but males are predominantly affected as seen in our study as well [7]. Contrary to reports in the literature indicating a higher prevalence of this mutation among Asian populations, it appears to be less common among Sri Lankans, particularly among the Sinhalese. Only three out of the 68 (4.4%) Sinhalese patients carried the mutation whereas two (brothers) out of the nine Tamil (22.2%) and three out of the 13 Moor patients (23.1%) carried the mutation. It is not scientifically valid to estimate prevalence for the Tamil and Moor groups in view of the small number in the present study. However, Asian populations where this mutation has been predominantly observed are either Moors or Indians [5, 7, 8]. Thus it is possible that the lower prevalence of *GHRH-R* codon 72 mutation seen in the present study is due to its lower prevalence among the Sinhalese who comprised the majority of the study group. Thirteen children including five with the mutation were born to consanguineous parents. Six of our patients from three families had a family history of GHD. Siblings from two of these families carried the codon 72 mutation, one set being from a consanguineous union.

The lean phenotype seen in GHD Type 1B has been reported in 78% of children with codon 72 mutation in the *GHRH-R* gene from Western India and Sindh Province of Pakistan [8]. We had noted that the majority of our patients with GHD were lean and petite unlike the commonly accepted phenotype of GHD which prompted us to do molecular genetic analyses in our patients. The 'leanness' of the patients was demonstrated anthropometrically as the BMI SDS, a value of <-2 being categorised as 'wasted'. The ideal method of determining 'leanness' is by assessing the body composition which could not be done at the time these children were diagnosed and was not applicable at the time of study as they were already on treatment which was started following the diagnosis. Five of the seven patients (71%) with the codon 72 mutation in whom anthropometric data were available had a BMI SDS <-2 whereas 38 (45.78%) of the mutation negative patients also had a similar BMI SDS although the association was not significant (p>0.05). Lack of statistical significance was perhaps due to the small number of mutation positive patients in our cohort. Although mutations at other sites of the *GHRH-R* gene are rarer than at codon 72, possibility of codon 72 mutation negative patients having other mutations resulting in a deficiency of GHRH action cannot be excluded.

It has been shown that only 3.7% of patients carry a *GHRH-R* mutation in a large cohort of children with GHD from multiple ethnic groups [9]. Fifteen patients from seven pedigrees carried a mutation in the *GHRH-R* gene and among them codon 72 mutation was observed in eight patients from three pedigrees, two Asian and one Somalian. Another two patients from an Asian pedigree were compound heterozygotes for the codon 72 mutation and a novel mutation, p.R161W (c.481C_T). A study from the UK, did not find any GHRH-R mutations in IGHD patients when Mediterranean, Asian, African, and Semitic descent were excluded [13]. *GHRH-R* mutations were not observed or were rare in several other populations. Mutations were not found among Dutch and Argentinean children with IGHD and were found only in three out of a cohort of 127 Japanese children, 14 with IGHD and 113 with idiopathic short stature [14-16]. Thus the lower prevalence of *GHRH-R* codon 72 mutation observed by us is not unexpected.

Conclusions

This is the first report to demonstrate the *GHRH-R* codon 72 mutation in Sri Lankan patients with confirmed growth hormone deficiency. The mutation was seen in 8.8% of these patients. The lean phenotype was not

necessarily associated with *GHRH-R* codon 72 mutation in our patients. Ours is the largest cohort of such children in Sri Lanka and for uniformity of management and follow up we did not include children who were treated elsewhere.

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Conflicts of interests

There are no conflicts of interest.

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