Case Report

Molecular characterization in a case of isolated growth hormone deficiency and further prenatal diagnosis of an unborn sibling

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Familial isolated growth hormone deficiency (GHD) type 1 is characterized by an autosomal recessive pattern of inheritance with varying degrees of phenotypic severity. We report a proband, with isolated GHD (IGHD) with very early growth arrest and undetectable levels of GH. Homozygous complete deletion of the *GH1* gene was identified by real-time/quantitative polymerase chain reaction (RT/q-PCR) and confirmed by an independent molecular genetic method; the multiplex ligation-dependent probe amplification (MLPA) technique. Prenatal diagnosis was offered for the subsequent pregnancy in the mother of our proband. Identical heterozygous deletion of the *GH1* gene was detected in both parents. The fetus had a similar homozygous deletion of the *GH1* gene. We thus report a unique case with a confirmed mutation in *GH1* gene in the proband followed by prenatal detection of the same mutation in the amniotic fluid which to our knowledge hitherto has not been documented from India.

Key words: Antenatal diagnosis, GH1 deletion, isolated growth hormone deficiency

Introduction

Isolated growth hormone deficiency (IGHD) has an incidence of 1:4,000 to 1:10,000 live births. Only 11-18% of these cases have an identifiable mutation and familial occurrence has been reported in 3-30% patients.[1,2] Having an autosomal recessive inheritance, IGHD type 1 is more common in consanguineous marriages. Some genetic variants of IGHD are associated with a phenotype of evolving multiple pituitary hormone deficiency.[3] It is useful to have a genetic diagnosis to get an insight into the expected response to GH therapy and to determine if any other endocrine abnormalities are likely to occur as a part of the spectrum of the disorder. It is also necessary to know the mutation status in the proband and parents to determine the exact mode of inheritance. Phenotypic severity and poor response to therapy associated with some genetic variants of IGHD thus justifies prenatal diagnostic workup of the fetus in a high-risk pregnancy.

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Thus, objective of our study was to detect if there was a prenatally diagnosable genetic cause of IGHD in the unborn sibling of a child who was clinically GHD. Specific aims were to: (i) Identify genetic cause of GHD in the (proband). (ii) If detected in the proband, determine if it was inherited from one/both parents. (iii) To check for presence of same in amniotic fluid.

Case Report

The proband had presented at 1 year with severe short stature. He was born of a consanguineous marriage and belonged to an inbred Muslim family. Frontal bossing, dysmorphic face with hypoplastic features, micropenis, and severe but

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proportionate short stature (height for age z score -9.6)[4] were suggestive of GHD. Clonidine stimulated GH concentrations were undetectable (<0.1 ng/mL). Response to GH therapy was excellent and at present he continues to take GH.

Parents of the proband presented for prenatal diagnosis for their next child at 13-14 weeks of gestation. After a proper pretest counseling and patient consent, amniocentesis procedure was performed at 16 weeks to obtain the amniotic fluid sample for molecular genetic testing.

Molecular genetic methods

Since a clinical diagnosis of IGHD had been made, known genes for IGHD for which testing was available were assessed, that is, a total of 17 amplicons of the GH releasing hormone-receptor (GHRHR) and *GH1* genes were tested. Vitamin D receptor (*VDR*) gene was used as a control. Real-time/quantitative polymerase chain reaction (RT/q-PCR) analysis which looks for changes in copy numbers in the patient compared to a control sample was carried out on genomic DNA of proband extracted from 200 μL of peripheral blood (Bioneer kit, Korea).

DNA was further tested using an independent molecular method, multiplex ligation-dependent probe amplification (MLPA). MLPA analysis was performed using the SALSA MLPA P216-A2 GHD Probemix (MRC-Holland, Netherlands) as per the manufacturer's instructions. The mix included MLPA probes spanning the *GH1, PROP1, POU1F1, GHRHR, HESX1, LHX3, LHX4* genes. Similar analysis was also carried out on the parents' samples.

Ten milliliter of the amniotic fluid sample was resuspended in 250 μl of phosphate buffered saline. Genomic DNA was isolated and used for analysis of the *GH1* gene by RT/q-PCR and MLPA (as described above).

Results

RT/q-PCR followed by gel electrophoretic analysis in the proband showed absence of amplification of all five *GH1* exons; however, expected amplification was seen in healthy control [Figure 1a and b], suggesting a homozygous deletion of all exons of the gene.

RT/q-PCR specificity was confirmed by melt curve analysis (data not shown).

MLPA analysis [Figure 2] showed complete lack of *GH1* amplification in the proband; whereas, it was in reduced intensity in both parents.

ΔCt values obtained on PCR analysis showed very low amplification in the proband, suggesting a complete deletion of *GH1* gene. Whereas, data of parents suggested a heterozygous deletion [Table 1].

Amniotic fluid sample also showed markedly reduced copy numbers of the *GH1* gene on RT/q-PCR; suggesting complete deletion of gene in the fetus. ΔCt values were similar to that of the proband [Figure not shown, data

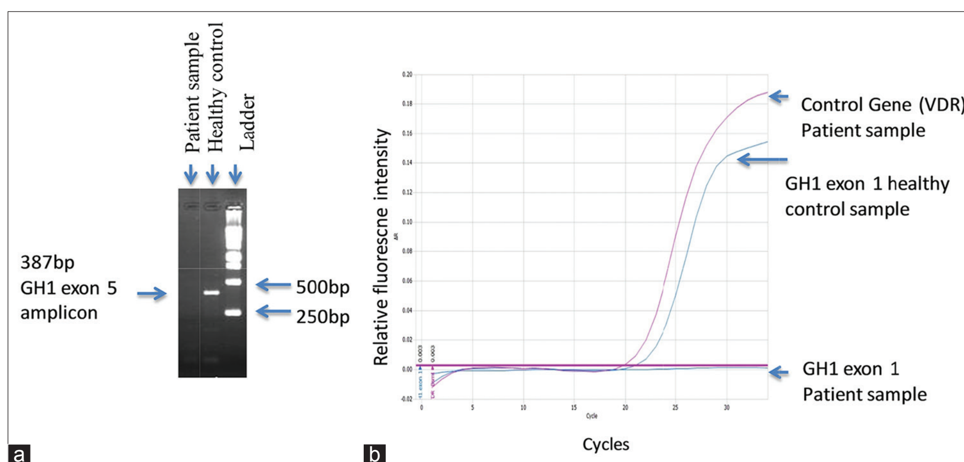

Figure 1: (a) Gel electrophoretic analysis of polymerase chain reaction (PCR) products of growth hormone (GH) 1 exon 5: Expected amplification is seen in healthy control. There was no amplification in the proband. (b) Real-time SYBR Green PCR of GH1 exon 1. Healthy control showed expected amplification. No amplification was seen in the proband's sample. Amplification of the *VDR* gene used as the control gene in the patient's sample showed normal amplification

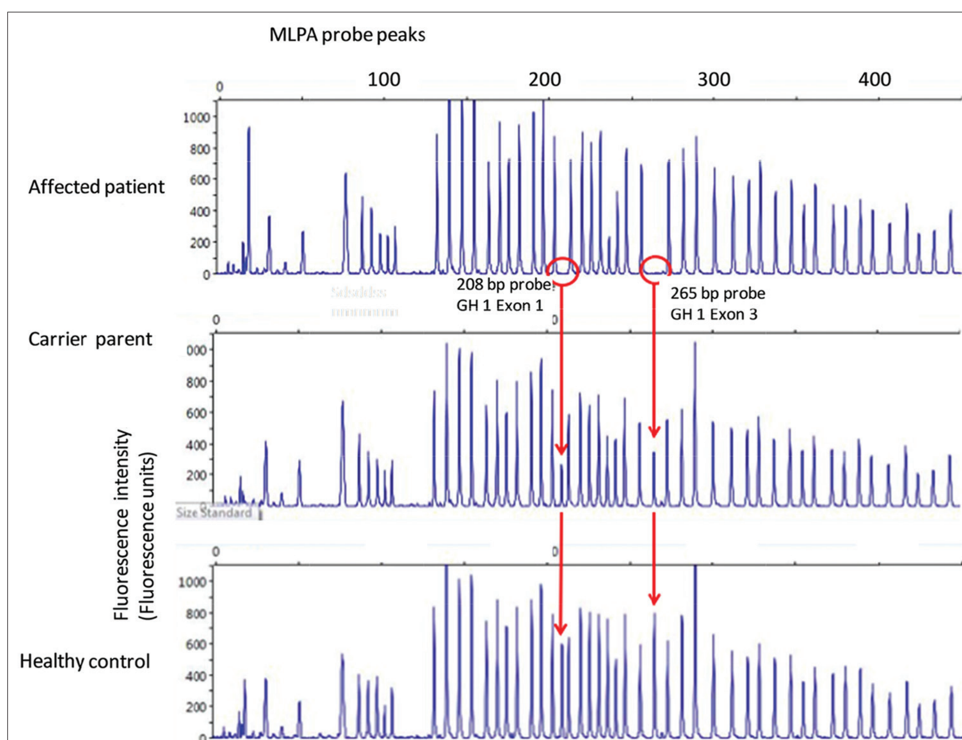

Figure 2: Multiplex ligation-dependent probe amplification (MLPA) analysis of proband sample along with father's sample (maternal data not shown). The GH1 specific amplicons (208 base pairs (bp) and 265 bp) are absent in the proband samples, at reduced intensity (nearly half normal indicating a heterozygous state) in the parental sample, and at full intensity in the control sample from a healthy male. (MLPA analysis of fetus not demonstrated graphically)

Table 1: Quantitative (real-time) SYBR green polymerase PCR on growth hormone 1 gene and the human vitamin D receptor gene

Sample	GH1 Ct	VDR Ct	Δ Ct
Mother	22.22	21.87	0.34
Father	22.27	22.12	0.15
Proband	28.25	21.82	6.43
Amniotic fluid	25.39	23.25	2.14

Cycle threshold (Ct): Fluorescence signal inflection points, DCt values: Difference between the GH1 and VDR Ct values. Each probe generates an amplification product of unique length that can be detected by capillary electrophoresis. Comparison of the relative sizes of the fluorescent peaks from the target probes with reference probes coamplified during the reaction, using the analysis software, allows the relative abundance of the target regions to be quantified. The proband showed a DCt value of 6.43 for GH1, that is, a 200-fold difference in copy number as compared to the *VDR* gene, indicating a failure to amplify the *GH1* gene within the expected normal range. A DCt value of 0.35 and 0.15 was seen in the mother and father, respectively (approximately two-fold difference in copy number) indicating slightly reduced amplification of the *GH1* gene, indicating a heterozygous deletion of GH1 in both parents

in Table 1]. Findings were independently confirmed by MLPA. All test controls (positive and negative) performed within expected range.

Discussion

We report here genetic analysis of a family in which proband had a homologous complete deletion of *GH1* gene (demonstrated by RT/q-PCR, confirmed by gel electrophoresis and MLPA), manifested at an early age, had severe phenotype of GHD, and showed good response to GH therapy. Parents were heterozygous carriers of the same deletion suggesting autosomal recessive inheritance [Figure 3].

Both parents are heterozygous carriers of the GH1 deletion. The proband and unborn fetus is homozygous for the same deletion.

Our data suggest that the fetus also had homozygous deletion of the *GH1* gene. All PCR and MLPA data were in concordance. MLPA is an extension of PCR that allows highly multiplexed detection of relative copy number variations of target sequences in a fast, reliable, and cost-effective manner.[5]

Mutations in patients with GHD are more likely to be identified in familial cases (34%) and in patients with a height Z score ≤ -4.5 (20%).[1,6,7] Genetic testing is thus strongly indicated in consanguineous marriages, those with positive family history and severe phenotype. *GH1* deletions result in complete block in GH synthesis, hence the phenotype is severe and anti-GH antibodies

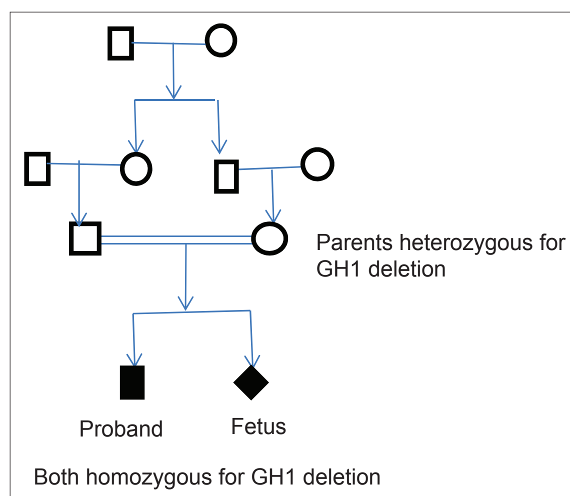

Figure 3: Family tree both parents are heterozygous carriers of the GH 1 deletion. The proband and unborn fetus are homozygous for the same deletion

develop during recombinant GH therapy.[8] Mullis and Brickell have reported antenatal diagnosis of two cases of *GH1* deletions in two at-risk pregnancies, using the PCR.[9] To the best of our knowledge, this is the first Indian report of antenatal diagnosis of *GH1* deletion.

To conclude, we report a family where homozygous *GH1* deletion was confirmed in a suspected case of IGHD followed by successful prenatal diagnosis for the subsequent unborn sibling of the proband. IGHD type 1 was confirmed by heterozygous status of both parents.

Treatment with GH is available, though still quite expensive in India. Therefore in these circumstances, antenatal diagnosis provides an opportunity for appropriate genetic counseling and early detection.

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