Case Report

A novel variant c.97C>T of the Growth Hormone Releasing Hormone Receptor gene causes isolated growth hormone deficiency type Ib

Short title: Novel c.97C>T variant of GHRH receptor

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What is already known on this topic?
Isolated growth hormone deficiency (IGHD) is a sporadic disease with insufficient or deficient production of growth hormone (GH). IGHD type Ib is caused by mutations to either the GH-1 gene or to the GHRH-R gene.

What this study adds?
We report a previously undescribed genetic defect, the c.97C>T variant of the GHRH-R gene, which results in severe growth retardation - almost growth arrest - in homozygous state. The present case provides new data on genetic causes of isolated growth hormone deficiency type Ib and describes the phenotype of the novel mutation.

Abstract
Congenital isolated growth hormone deficiency (IGHD) type Ib is an autosomal recessive genetic condition caused by mutations of GH1 or the GH releasing hormone receptor (GHRH-R) gene. Affected subjects present symptoms of GHD with low but detectable levels of GH, short stature and responsiveness to GH therapy. We describe a 13-month old girl with severe growth failure who showed a low hGH in response to 2 hGH provocative tests and a modest increase of IGF-I to an IGF-I generation test. Whole exome sequencing revealed a novel homozygous variant of the GHRH-R gene (c.97C>T), creating a premature stop codon. Administration of recombinant human GH improved linear growth. This is the first report of a c.97C>T mutation of the GHRH-R gene.

Keywords: Congenital isolated growth hormone deficiency, growth hormone releasing hormone receptor, c.97C>T, failure to thrive, short stature

Introduction
Isolated growth hormone deficiency (IGHD) is a sporadic disease with a prevalence ranging from 1:3.480 to 1:10.000 live births (1). It is defined as an insufficient or deficient production of growth hormone (GH) by the pituitary gland. Its complex etiology involves a spectrum of hypothalamic
defects, pituitary abnormalities or combined conditions, which can be structurally detected by brain imaging in only 26.8% of the affected population (2). Familial IGHD is classified into four distinct types with different clinical manifestation and inheritance patterns. The two most frequent types of IGHD are types la and lb, characterized by an autosomal recessive trait; type II is transmitted as an autosomal dominant defect while type III appears with an X-linked inheritance pattern. Type Ia IGHD presents as an entire GH-1 gene deletion with undetectable serum GH levels, extremely short stature and possible development of anti-GH antibodies after recombinant human growth hormone administration (3-4). Type Ib IGHD presents a milder phenotype, caused by mutations to either the GH-1 gene or to the gene with low but detectable levels of serum GH, short stature and a positive response to GH therapy with immunologic tolerance (5). Type II IGHD patients also may present low serum GH levels without anti-GH antibodies development. IGHD-type III has been associated with occasional agammaglobulinaemia (6).

The GHRH receptor (GHRH-R) gene is located on the short arm of chromosome 7. A number of mutations in the specific locus of GHRH-R gene, has till now been reported in IGHD type Ib subjects, leading to loss of the receptor function and thus to growth failure. We present a novel mutation of the GHRH-R, leading to IGHD type Ib in a 13-months old Greek girl, the youngest patient reported so far.

Case report
A 13-month old girl was admitted to our Department due to failure to thrive. She was the second child of a phenotypically healthy unrelated parents, whose height was 190 cm and 175 cm, respectively. Ethical review board approval and informed consent from both parents of the proband participating in this paper was obtained in accordance with the national laws. The patient was the product of 37 weeks gestation. During the 4th-8th gestational week, the mother experienced vaginal bleeding. Intrauterine growth retardation was diagnosed in the 8th gestational week due to placental insufficiency. Additionally the mother admitted a smoking habit during the entire pregnancy. The newborn was asymmetrical and small for gestational age, with a birth weight of 2420 gr (<3rd percentile, z-score: -1.93), and 44 cm length (<3rd percentile, z-score: -2.76) (Figure 1). Head circumference was 34.5 cm (70th percentile, z-score: 0.52). She was partially breast-fed during the first 30 days of life. Due to the infant’s unwillingness to take the formula milk she was admitted to the pediatric gastroenterology department where a 24-hours nasogastric tube was placed at the age of 9 months and hypercaloric oral supplements were administered, without significant effect on body weight gain (Figure 1).

On physical examination, at 13 months of age, the infant was small and skinny, with a nasogastric feeding tube - not resembling the obese GH deficient neonates-, her length was 60 cm (<<3rd percentile, z-score: -6.03) and her weight 5470 gr (<<3rd percentile, z-score: -4.35). Head circumference was 45 cm (40th percentile, z-score: -0.27) and head shape was triangular with open fontanels. Hair was very sparse and ears were low set. Nasal bridge was hypoplastic and dental development was significantly retarded (one tooth). Motor milestones were delayed; she was able to sit but not to stand. Systematic clinical examination of heart, lung and abdomen did not reveal abnormal findings. Complete blood count, haemoglobin levels, glucose concentrations as well as renal function were within normal range for her age. Karyotype analysis showed a normal female of 46 XX. Thyroid and adrenal hormones levels were normal. Serological indexes for celiac disease or food allergy were negative. Serum GH response to clonidine, glucagon and arginine stimulation tests revealed low responses with peak GH value of 4.77 ng/ml, demonstrating IGHD (Table 1). An IGF-I generation test after administering GH in a dose of 33 µg/kg for 4 consecutive days showed low IGF-I levels with a modest response (Table 1). After 12 months of GH treatment, serum IGF-I rose to 23 ng/ml. Bone age was 2 months at the chronological age of 13 months. Magnetic resonance imaging of the brain revealed normal pituitary gland and hypothalamus.

At the chronological age of 19 months the patient was administered GH on a starting dose of 0.28 mg/kg/week subcutaneously. After ten months, GH dose was increased to 0.35 mg/kg/week. At the age of 22 months she started to walk and bone age improved. At the chronological age of 24 months she presented a 12 months phalangeal and a 9 months carpal bone age. After 10 months of medication she gained 7 cm in length (8.14 cm/year), 300 gr in weight and she exhibited a head circumference augmentation of 2.2 cm. In one year of treatment (chronological age of 31 months) the patient
presented with 73.5 cm length (<3rd percentile, z-score: -5.2), 6100 gr weight (<3rd percentile, z-score: -5.65) and 48 cm head circumference (50th percentile, z-score: -0.02) (Figure 1).

Due to the facial features of the patient, Silvel-Russell syndrome has been suspected. The absence of the clinical criteria of Price et al. (7) along with a deletion/duplication analysis with array genomic hybridization, excluded Silvel-Russell syndrome. Additionally, intrauterine growth retardation along with facial characteristics and delayed eruption of teeth, could suggest a possible 3M syndrome. Triple whole exome sequencing (WES) of the affected girl and parents (CentoXome GOLD®) using Illumina technology was performed. No mutation on CUL7, OBSL1 or CCDC8 genes, the causatives for 3M syndrome loci, were found. A novel homozygous nonsense variant in the GHRH-R gene, the c.97C>T (p.Gln33*) was detected. The observed variant creates a premature stop codon, and is classified as likely pathogenic-class 2 variant. Parental genotyping detected the novel variant in the mother in a heterozygous state, but it was not found in the father. It is suspected that a large deletion not detectable by whole exome sequencing in the paternal allele is present. The detected c.97C>T variant of the GHRH-R gene has never been reported before and it not listed as novel in the CentoMD.

Discussion

The present report describes the youngest patient with a so far unknown GHRH-R mutation, in an infant girl of Greek origin with a clinical appearance resembling small for gestational age (SGA) rather than congenital GHD (8, 9). Striking differences were the skinny appearance rather than the obese and the low IGF-I response to an IGF-I generation test. These two unexpected findings probably relate IGF-I to caloric insufficiency caused by the placental insufficiency (10) and possibly by the smoking habit of the mother.

One of the causes for congenital IGHD is GHRH-R gene defects. Their detection is following a gradually increasing trend (11). Nowadays more than thirty-three mutations in the GHRH-R gene have been acknowledged as responsible for impaired GHRH-GH-IGF-I axis function, whereas no mutations in the GHRH-R gene have been reported. In the large majority of them, an autosomal recessive model of inheritance is being followed (12). Mutations of GHRH-R, classified into six different types, cause defective GHRH functionality (13). Null-type GHRH-R mutations lead to unmeasurable IGF-I levels, are accompanied by mild ocular disorders (14). Missense GHRH-R variants—such as p.G369V or p.T257A—result in partial loss of receptor function due to defective ligand binding and milder phenotypes, occasionally accompanied by hypoglycemia (15). Splicing site mutations of untranslated and coding regions reveal gross indels with loss of 5' regulatory/exon 1 region, leading to fully impaired GHRH-R expression (12). Other splice-disrupting single nucleotide polymorphisms like intronic mutations lead to instability of the produced mRNA, truncated GHRH-R and autosomal recessive IGHD (16). Nonsense type mutations lead to loss-of-function changes (17), whereas functional variants of the GHRH-R promoter affect promoter activity and thus decrease expression of the receptor gene (18).

Hereby, we present a novel previously undescribed GHRH-R gene mutation, c.97C>T (p.Gln33*) in homoygous zyosity, in a child with IGHD type Ib. Clinical and biochemical phenotype of the affected individual comprises severe short stature, low weight gain, low maximum GH values during provocation testing, inadequate response in IGF-I generation testing, normal brain imaging and growth acceleration after GH therapy. The reported mutation creates a premature stop codon and thus it signals the termination of translation of the relevant messenger RNA. Defective translation of the gene results in a shorter encoded protein and thus an impaired form of GHRH receptor. The novel mutation affects the receptor in terms of sequence and structure, probably leading to the inhibition of GHRH binding to its receptor and thus to disruption in GH secretion signaling. According to the recommendations of the American College of Medical Genetics and Genomics, the novel mutant classified as likely pathogenic class 2. Since the variant was detected in the maternal DNA in heterozygous state, but not in the paternal genome, precise pattern of inheritance can not be fully declared. A suggested large deletion in the exact region of the paternal GHRH-R locus, could explain the inability to detect the mutation via father WES analysis. Nevertheless, it could be assumed that the hereby presented p.Gln33* variant presents an autosomal recessive inheritance trait, on grounds of the healthy unaffected heterozygous mother.

Intrauterine growth restriction is closely associated with placental quality, functionality and therefore adequacy. Multiple layers of associations have been acknowledged at the causality investigation of
fetal growth restriction and SGA offsprings. In utero exposure to tobacco constitutes a known risk factors for both conditions. From a meta-analytic approach, even smokeless tobacco use during pregnancy is associated with low birth weight (19). Exposure of offspring to tobacco metabolites through maternal milk during the infantile growth is also questioned (20). Nevertheless, cohort studies have provided evidence that maternal smoking during pregnancy or early infantile life exert long-term negative effect on growth (21). The presented case constitutes a paradigm of mixture between nature and nurture. Apart from the detected defect in GHRH-R gene sequence, in utero environment and after birth conditions have contributed to the phenotype. Synergistic effect of genetics and epigenetic conditions have to be explored in the future.

In conclusion we report a novel homozygous c.97C>T (p.Gln33*) GHRH-R mutation determined in a Greek infant girl with IGHD. Heterozygosity of the reported variant was not associated with pathological phenotypes in the unaffected family members c.97C>T.

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Conflicts of interest
The authors declare no conflict of interest.

Authorship contributions
Concept: Assimina Galli-Tsinopoulou
Design: Assimina Galli-Tsinopoulou, Eleni P Kotanidou, Zvi Laron
Data Collection or Processing: Eleni P Kotanidou, Aggeliki N Kleisarchaki, Rivka Kauli
Analysis or Interpretation: Rivka Kauli, Assimina Galli-Tsinopoulou, Zvi Laron
Literature Search: Eleni P Kotanidou, Aggeliki N Kleisarchaki, Assimina Galli-Tsinopoulou

References

Table 1. Growth hormone provocation and IGF-I generation test values

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<th>Time (min)</th>
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<th>Arginine (ng/ml)</th>
<th>IGF-I Generation Test</th>
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Figure 1. Growth chart for weight-for-age, height-for-age and head circumference-for-age (Anthro WHO software) along with a patient photo.