TITLE

Familial Isolated Growth Hormone Deficiency Due to A Novel Homozygous Missense Mutation in the Growth Hormone Releasing Hormone Receptor Gene: Clinical Presentation with Hypoglycaemia

Huseyin Demirbilek[1,2,3] Sophia Tahir[1], Riza Taner Baran[3], Maha Sherif[1], Pratik Shah[1,2], Mehmet Nuri Ozbek[3], Nebahat Hatipoglu[4], Ahmet Baran[4], Ved Bhushan Arya[1,2], Khalid Hussain[1,2]

[1]Developmental Endocrinology Research Group, Clinical and Molecular Genetics Unit, Institute of Child Health, University College London, WC1N 1EH, [2]Department of Paediatric Endocrinology, Great Ormond Street Hospital for Children, NHS Foundation Trust, WC1N 3JH London, UK

[3]Department of Paediatric Endocrinology, Diyarbakir Children's State Hospital, 21100 Diyarbakir, Turkey

[4]Department of Radiology, Diyarbakir Children's State Hospital, 21100 Diyarbakir, Turkey

OBJECTIVES

Familial isolated growth hormone deficiency (IGHD) may result from mutations in the human GH gene (*GH1*), GHRH receptor gene (*GHRHR*), and more rarely from heterozygous mutations in *HESX1* or *SOX3*. Mutations in the growth hormone releasing hormone receptor (*GHRHR*) gene are a relatively rare cause of IGHD. Aim of the present study was to understand the biochemical basis of hypoglycaemia in the index case and the molecular basis of severe short stature in a large consanguineous family with IGHD.

METHODS

Patients :The index case presented with a hypoglycaemic (blood glucose 1.2 mmol/l) convulsion at the age of 2.3 years. He was born to consanguineous normal height parents at 40 weeks gestation with birth weight of 2.2 kg (<-2 SD). There was no significant medical history. On examination, his weight was 10 kg (-2.68 SD) and height was 73.2 cm (-5.01 SD). Family history revealed three siblings with severe short stature (Table 1). Hypoglycaemic screen showed undetectable serum insulin level (< 2mU/l), a satisfactory ketogenic (β-hydroxybutyrate 1.7mmol/l) and serum cortisol response (35.7µg/dl), and an unsatisfactory GH response (0.5ng/ml). Measurement of serum insulin-like growth factor 1 (IGF1) and IGF binding protein 3 (IGFBP3), and GH provocation testing (Clonidine and L-Dopa stimulation tests) confirmed GH deficiency. Other investigations (serum ammonia, lactate, carnitines, acylcarnitines and serum amino acids) looking for other causes of hypoglycaemia were normal. Following the diagnosis of the index case, other members of the family (family 1) were evaluated for GH deficiency (Table 1). Interestingly, another consanguineous family very closely related to this family (Figure 1) also had multiple members with short stature without associated clinical features (Table 1).

All other affected members underwent baseline investigations for short stature including full blood count, erythrocyte sedimentation rate, blood biochemistry, liver and renal function tests, coeliac screen, thyroid function tests, serum IGF1 and IGFBP3. All these investigations were within normal range apart from serum IGF1 and IGFBP3, which were significantly below the normal range for age and sex (Table 1). Monitoring of growth over 6 months revealed low height velocity in all affected members (Table 1). All affected individuals showed a more than two-fold improvement in pre-treatment height velocity in response to recombinant GH therapy (0.03 mg/kg/d) (Table 1). Hypoglycaemia in the index case resolved after the GH was started.

Hormonal Studies and Pituitary imaging: All affected members of these two families underwent two GH provocation tests (L-Dopa and Clonidine tests) on separate days according to standard guidelines. Periodic assessment of other pituitary functions such as early morning cortisol, thyroid functions, and gonadotropins was also performed until the confirmation of underlying genetic diagnosis. An MRI scan of the pituitary gland of each GHD patient was independently reviewed by two experienced neuro-radiologists for anterior pituitary hypoplasia and position of posterior pituitary bright spot. Measurements of anterior pituitary were compared to age adjusted normal values.

Homozygosity mapping: Homozygosity mapping (HZM) was performed on two affected and one unaffected individual from each of the two families to identify potential candidate genes. The Illumina microarray platform was used for the genotyping following the Infinium HD Ultra Assay protocol (Rev B, 2010, Illumina Inc, San Diego, USA). The copy number variation and loss of heterozygosity data was generated using the Illumina Genomestudio software (cnvPartition v3.1.6, Illumina). The BEDtools were used for subsequent analysis of the homozygosity mapping data

GHRHR sequencing: All the 13 exons of *GHRHR* gene were PCR amplified and sequenced in two affected patients (one from each family) using the BigDye Terminator v 1.1 Cycle sequencing kit (Applied Biosystems, Foster City, California) according to manufacturer's instructions. Any deviations from reference sequence were checked in dbSNP database, 1000 Genomes Project and Exome Variant Server. The identified variant was subsequently screened in other family members to analyse for co-segregation with disease phenotype. In silico analysis of the identified variant was performed using various software programs such as PolyPhen 2, MutationTaster and Provean. The protein BLAST tool from the NCBI (National Centre for Biotechnology information) website was used to analyse the conservation of the amino acid of interest amongst species. The UniProt database was used to localise the mutation within the protein.

RESULTS

Hormonal Studies and Brain Imaging: The GH provocation testing in all affected individuals confirmed GH deficiency (Table 1). Pituitary MRI showed anterior pituitary hypoplasia in all affected members from Family 1, whereas the anterior pituitary was normal in size in two and at the lower end of the normal range in one patient (-2 SDS) from Family 2 (Table 1 and Figure 2). The posterior pituitary gland was eutopic in all affected patients (Table 1).

Homozygosity Mapping: Homozygosity mapping identified two large shared homozygous regions on chromosome 7 in four affected individuals which was not shared with two unaffected individuals. The *GHRHR* gene was located in one of these regions.

GHRHR gene sequencing and In silico analysis: Sequencing identified a novel homozygous missense c.190T>G (p.C64G) mutation in exon 3 of the *GHRHR* gene in all affected individuals (Figure 1). This mutation was not present in the Human Gene Mutation Database (HGMD), dbSNP database, 1000 Genome or Exome Variant Server database. Sequencing of the other family members showed co-segregation of the identified variant with the disease phenotype. In silico analysis with PolyPhen-2, MutationTaster and Provean tool predicted the mutation to be probably damaging (score 0.997), disease causing and deleterious (score -10.562) respectively. Pfam predicted an extracellular hormone receptor domain (PF02793) between amino acid positions 53 and 112 in GHRHR. This region has 4 conserved cysteine residues, which are predicted to be important in the formation of disulphide bonds, and hence necessary in maintaining protein stability and structure. The affected cysteine (p.C64G) residue in our patients is also highly conserved throughout species. The mutation resulted in substitution of conserved amino acid cysteine with glycine at position 64 (Figure 1). Therefore this mutation likely disrupts the structure and function of GHRHR protein or being present in a hormone receptor domain, its binding ability to other proteins.

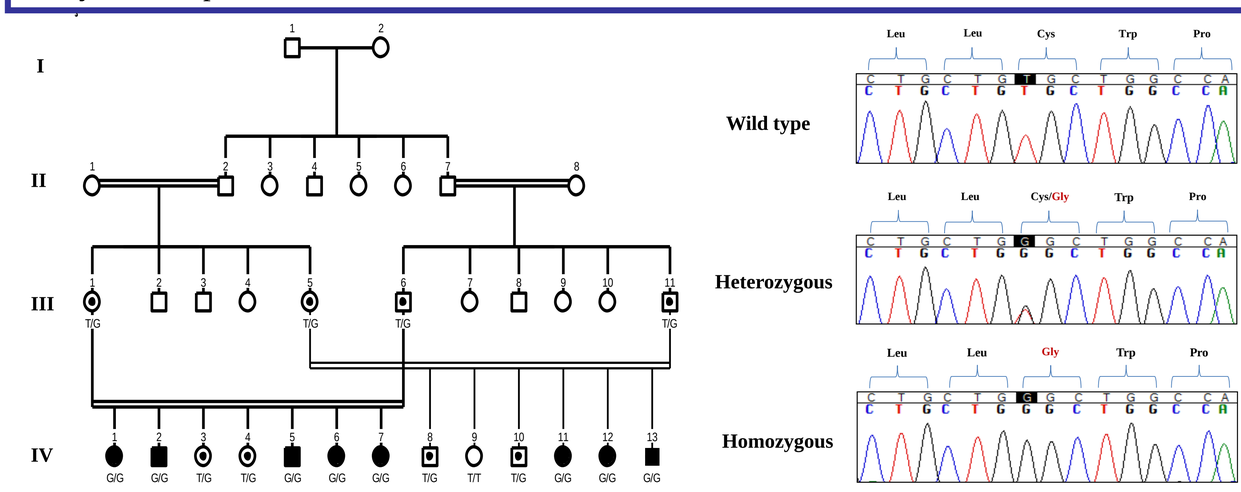

Figure 1. Pedigree and sequencing results of families with IGHD; Circles indicate females; squares indicate males; black fill indicate homozygous for mutation; white fill indicate homozygous for wild type allele and white fill with central black dot indicate heterozygous

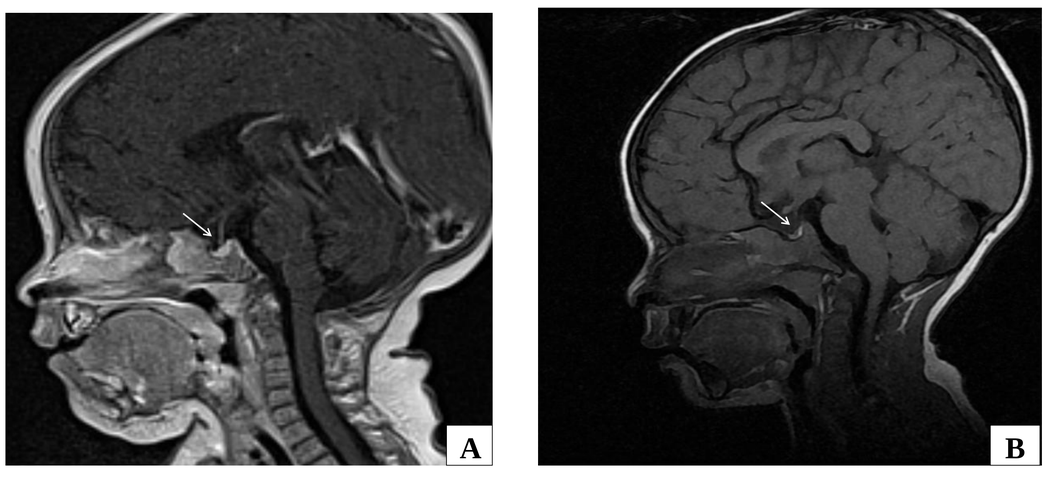

Figure 2: Anterior pituitary hypoplasia and normal pituitary stalk in the youngest (5 months) patient (A) and more severe anterior pituitary hypoplasia and thin pituitary stalk in her older 4.5 year-old sibling(B).

Table1. Clinical and biochemical characteristics and anterior pituitary MRI imaging results of patients with IGHD

Patient	Sex	CA (year)	BA (year)	Ht (cm)	Ht SDS	Weight (kg)	Mother's Ht (cm)	Father's Ht (cm)	IGF1 (ng/ml)	IGFBP3 (ng/ml)	Peak GH-L (ng/ml)	Peak GH-C (ng/ml)	APHt** mm (SDS)	PP	GH dose (mg/kg/d)	HV_0 (cm/year)	HV_1 (cm/ year)
Subfamily-1																	
IV.1	F	0.4	0.2	56.5	-3.23	5.5	153.5	165.3	(<-2SD)	<250	0.3	1.4	2.4 (-2.2) (3.5±0.5)	n	0.03	NA	NA
IV.2¥	M	2.3	0.5	73.2	-5.01	10	153.5	165.3	(<-2SD)	<250	*0.5	0.3	2.1 (-2.7) (4.0±0.7)	n	0.03	4.2	11
IV.5	M	4.5	2.3	84.4	-5.22	9.7	153.5	165.3	(<-2SD)	<250	0.3	0.9	1.8 (-3.1) (4.0±0.7)	n	0.03	2.6	13.2
IV.6	F	6.0	1.5	78.5	-8.14	8.8	153.5	165.3	(<-2SD)	<250	0.2	0.4	2.8 (-2.8) (4.5±0.6)	n	0.03	2.3	15.3
IV.7	F	8.6	3	78.5	-9.16	10.9	153.5	152.9	(<-2SD)	<250	0.1	1.7	2.5 (-3.3) (4.5±0.6)	n	0.03	2.2	15
Subfamily-2																	
IV.11	F	6.3	4	89.9	-5.97	12.6	149.3	167.2	24.7 (<-2SD)	396 (<-2SD)	0.25	1.28	3.3 (-2) (4.5±0.6)	n	0.03	3.2	9.6
IV.12	F	8.7	4.5	93.8	-6.41	13	149.3	167.2	24.7 (<-2SD)	423(<-2SD)	1.01	0.15	4.0 (-0.8) (4.5±0.6)	n	0.03	3.0	9.2
IV.13	M	9.3	4.2	98.7	-6.23	14.7	149.3	167.2	24.8 (<-2SD)	386(<-2SD)	0.75	0.78	4 (-0.8) (4.5±0.6)	n	0.03	3.1	11

F: Female; M: Male; CA - chronological age at presentation (year); Ht: Height; UD: Undetectable; GH: Growth hormone; IGF1: Insulin like growth factor 1 (ng/ml); IGFBP3: Insulin-Like Growth Factor Binding Protein 3 (ng/ml); GH-L: Peak GH level on L-dopa stimulation test; GH-C: Peak GH level on clonidine stimulation test; APHt: Anterior pituitary height (**Values given in parenthesis are mean±SD for age normal); SDS: Standard deviation score; PP: Posterior pituitary; HV_0: Height velocity (pre-treatment); HV_1: Growth velocity after rhGH therapy; MPH: Mid parental height; *Presented with hypoglycaemic convulsion GH level measured 0.5 ng/ml when blood glucose was 1.2 mmol/L. ¥Indicates index case

CONCLUSIONS

We describe a large consanguineous Turkish kindred with multiple members affected with a novel homozygous missense *GHRHR* mutation. The index case who presented with hypoglycaemic convulsion, is the first description of hypoglycaemia with *GHRHR* mutation to date. A marked AP hypoplasia was observed in all affected members from subfamily-1, whereas, affected patients from subfamily-2 had normal or borderline AP sizes. Two affected individuals from subfamily-1 displayed anterior pituitary hypoplasia at the very young ages of 0.4 and 2.3 years. Another interesting observation was the striking difference in auxological and biochemical severity between the two families despite identical *GHRHR* mutation. We suspect this difference in phenotype may be due to the effect of other unknown modifier genes.