# Partial Loss of Function of the GHRH Receptor Leads to Mild Growth Hormone Deficiency

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**Objective:** Recessive mutations in *GHRHR* are associated with severe isolated growth hormone deficiency (IGHD), with a final height in untreated patients of 130 cm ± 10 cm (−7.2 ± 1.6 SDS; males) and 114 ± 0.7 cm (−8.3 ± 0.1 SDS; females).

**Design:** We hypothesized that a consanguineous Pakistani family with IGHD in three siblings (two males, one female) would have mutations in *GH1* or *GHRHR*.

**Results:** Two novel homozygous missense variants [*c.11G>A* (p.R4Q), *c.236C>T* (p.P79L)] at conserved residues were identified in all three siblings. Both were absent from control databases, aside from pR4Q appearing once in heterozygous form in the Exome Aggregation Consortium Browser. The brothers were diagnosed with GH deficiency at 9.8 and 6.0 years (height SDS: −2.24 and −1.23, respectively), with a peak GH of 2.9 μg/liter with low IGF-1/IGF binding protein 3. Their sister presented at 16 years with classic GH deficiency (peak GH <0.1 μg/liter, IGF-1 <3.3 mmol/liter) and attained an untreated near-adult height of 144 cm (−3.0 SDS); the tallest untreated patient with *GHRHR* mutations reported. An unrelated Pakistani female IGHD patient was also compound homozygous. All patients had a small anterior pituitary on magnetic resonance imaging. Functional analysis revealed a 50% reduction in maximal cAMP response to stimulation with GHRH by the p.R4Q/p.P79L double mutant receptor, with a 100-fold increase in EC50.

**Conclusion:** We report the first coexistence of two novel compound homozygous *GHRHR* variants in two unrelated pedigrees associated with a partial loss of function. Surprisingly, the patients have a relatively mild IGHD phenotype. Analysis revealed that the pP79L mutation is associated with the compromise in function, with the residual partial activity explaining the mild phenotype. **(*J Clin Endocrinol Metab* 101: 3608–3615, 2016)**

The gene encoding the growth hormone releasing hormone receptor (*GHRHR*) is 15.51 kb in length and incorporates 13 exons on chromosome 7p14. It encodes a G-protein coupled receptor (423 aa) and is expressed on the somatotroph cells of the anterior pituitary (1). Its ligand GHRH, released from the hypothalamus, stimulates the synthesis and release of GH (encoded by *GH1*) upon binding in the presence of the pituitary-specific transcription factor POU1F1 (2, 3). GH in turn binds to receptors on the liver and generates IGF 1 and IGF binding protein 3 (IGFBP3), thereby promoting growth.

Consistent with their role in growth regulation, mutations in *GHRHR*, *GH1*, and *SOX3* are implicated in the etiology of isolated GH deficiency (IGHD) (4), and the

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Abbreviations: APH, anterior pituitary hypoplasia; GHD, GH deficiency; GHRHR, GH releasing hormone receptor; hCG, human chorionic gonadotrophin; IGFBP3, IGF binding protein 3; IGHD, isolated GH deficiency; MRI, magnetic resonance imaging; NR, normal range; rhGH, recombinant human GH; SDS, standard deviation score.

pathway was recently implicated in the GH deficiency (GHD) phenotype observed in the autosomal dominant disorder pseudohypoparathyroidism type 1b (5). Autosomal recessive mutations occurring in the *GHRHR* gene have been implicated in severe IGHD type 1B, also known as Sindh dwarfism (6, 7). Reported aberrations in *GHRHR* have included missense, splice (8), nonsense (9, 10), and microdeletion and promoter mutations (11, 12). Many have been shown to specifically affect cAMP production, for example *GHRHR* (p.K329E), which fails to show a cAMP response after treatment with GHRH (13). All mutations described to date have shown a complete loss of function.

Severe IGHD type 1B was initially described in pedigrees from the Indian subcontinent (14) and Brazil (15). Interestingly, the phenotype is usually not that of classic IGHD in that affected patients have minimal facial hypoplasia and no microphallus, but do manifest anterior pituitary hypoplasia (APH) on their magnetic resonance imaging (MRI) (3). However, growth failure is severe with proportionate dwarfism and pubertal delay, and biochemically, the patients have low GH and IGF1 concentrations with otherwise normal pituitary function. To date, reported height in untreated patients with a *GHRHR* mutation is on average 130 ± 10 cm (−7.2 ± 1.6 SDS) in males and 114 ± 0.7 cm (−8.3 ± 0.1 SDS) in females (16).

Previous studies in our cohort of IGHD patients (n = 224) revealed *GHRHR* mutations in 3.7% of cases (15 patients from seven pedigrees). All were familial cases, predominantly from the South East Asian community, manifesting severe growth failure with the vast majority showing APH on their MRI scan (7). In this manuscript, we report the presence of two homozygous variants in *GHRHR* in consanguineous pedigrees with a relatively mild GHD phenotype and present functional data that reveal the first partial loss of function mutation in *GHRHR*. Additionally, an independent patient with the identical variants was also identified, suggesting the presence of a founder effect.

## Materials and Methods

### Patients

DNA was extracted from blood samples taken from two consanguineous pedigrees with IGHD. Ethical committee approval was obtained from the Institute of Child Health/Great Ormond Street Hospital for Children Joint Research Ethics Committee and informed written consent was obtained from patients and/or parents.

### Direct sequencing analysis

Three siblings with IGHD from pedigree I and a separate patient from pedigree II were screened for *GH1* and *GHRHR* mutations. The coding region of these genes consists of five exons in *GH1* and 13 exons in *GHRHR*. These were amplified by PCR on an Eppendorf Thermocycler over 35 cycles, with primers designed using the Primer3 program (available at http://frodo.wi.mit.edu/primer3) flanking each of the exons in the coding regions of the genes. PCR products were treated with MicroClean reagent (Web Scientific, catalog no. 2MCL-10) according to manufacturer's instructions and then sequenced using BigDye v1.1 sequencing chemistry (Applied Biosystems) and analyzed on a 3730 × 1 DNA Analyzer (Applied Biosystems/Hitachi, catalog no. 625–0020). Details of the PCR conditions are available upon request, including the primer sequences, product sizes, and annealing temperatures. For any mutations identified, control databases were consulted as follows: Exome Variant Server (evs.gs.washington.edu/EVS/), 1000 Genomes (www.1000genomes.org), an in-house panel of 200 ethnically matched controls, and the Exome Aggregation Consortium (ExAC Browser) (http://exac.broadinstitute.org/).

### Molecular modeling

The RasMol prediction model database was used to build a three-dimensional annotated model of the GHRHR wild-type and mutant proteins, respectively, to analyze and compare protein folding and structure.

### Functional analysis

An expression vector was obtained encoding full-length wild-type *GHRHR* cloned into pcDNA3.1 (Source Bioscience). Detected mutations p.R4Q, p.P79L, and the double mutant p.R4Q/p.P79L were introduced into the sequence using the QuikChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies UK Ltd). Vectors were transfected into HEK293 cells (American Type Culture Collection) cultured in DMEM supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 1% nonessential amino acids at 37 C in a humidified 5% $CO_2$ incubator. Approximately $1 \times 10^6$ cells were transfected with 1.2 μg Glosensor 22F (Promega) and 1.2 μg GHRHR using Polyjet transfection reagent (SignaGen Laboratories) according to the manufacturer's instructions. Cells were plated in a white 96-well dish at a density of approximately 35 000 cells per well and the following day media replaced with Leibovitz's L-15 medium (Thermo Fisher Scientific) containing 2 mM luciferin (Promega). After equilibration at 25 C, the basal luciferase activity was measured on a Glomax luminometer (Promega) and cells were then stimulated with various concentrations of GHRH 1–44 (Bachem) and the luciferase response monitored approximately every 3 minutes for at least 60 minutes. Response to GHRH was calculated as the area under the curve for the period of measurement after correction for background activity from unstimulated cells.

## Results

### Patient phenotypes

#### *Patient IV.1*

The proband was a male born at term (birth weight, 3.6 kg) to a consanguineous Pakistani family (Figure 1A), and first presented at the age of 4 years with bilateral unde-

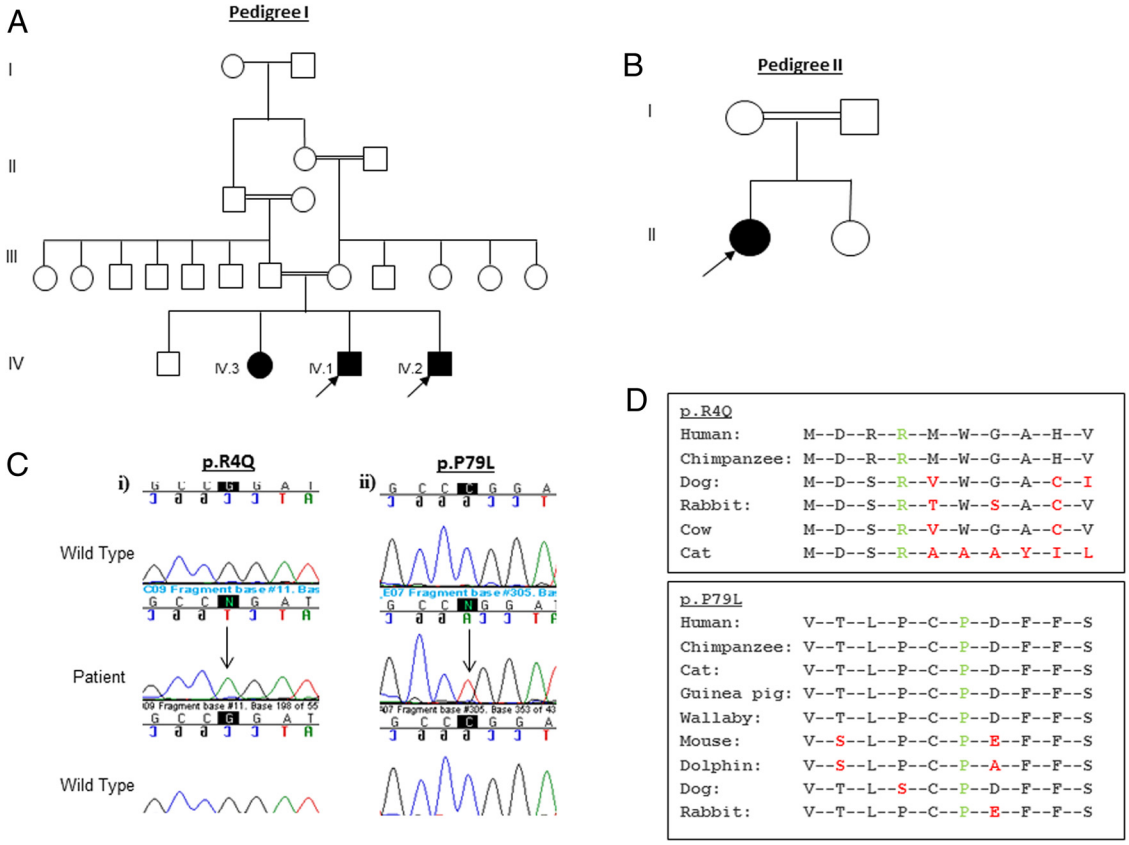


**Figure 1.** A, Consanguineous Pakistani pedigree with IGHD. This family tree shows two male probands in pedigree 1 and their affected sister (shaded black squares and a shaded circle, respectively). The double lines represent consanguinity, with the parents of the affected patients being first cousins. The generations within the family are indicated by roman numerals. (B) Pedigree II with IGHD. This family consists of one affected female (shaded black circle) and her unaffected sister, born to first-cousin parents. (C) Two *GHRHR* mutations associated with IGHD phenotypes. A novel homozygous missense mutation, c.11G>A causing a p.R4Q substitution, was identified in exon 1 ((i), shown as N′ and indicated by arrow) and a homozygous missense mutation; c.236C>T, causing a p.P79L substitution, was found in exon 3 ((ii), shown by N′ and indicated by arrow) in three siblings from pedigree I and in an unrelated female patient from pedigree II. (D) Highly conserved residues across multiple species. GHRHR protein sequences spanning both amino acids that are substituted in the patients. The p.R4 and p.P79 are represented in green and show high conservation between multiple species. Any spanning amino acid residues that differ from the reference human sequence are highlighted in red.

scended testes, micropenis, and a hypoplastic scrotum. There was no history of neonatal hypoglycemia or jaundice, he had no dysmorphic features, and at presentation his height was 100.7 cm (−0.73 SDS) with a weight of 14.8 kg (−1.09 SDS). At the age of 4.2 years, he had an acceptable testosterone response to a 3-day human chorionic gonadotrophin (hCG) stimulation test, rising from 0.4 to 4.8 nmol/liter and basal gonadotrophins in the prepubertal range (LH < 0.7 U/liter, FSH 1.0 U/liter). Following hCG stimulation, testes were bilaterally palpable; however, later examination revealed impalpable testes and he received a further 6-week course of treatment with hCG at the age of 7 years with a partial response and underwent bilateral orchidopexies at the age of 8.2 years. Between the ages of 4 and 7 years, he grew steadily with a growth velocity of 5.0 to 5.5 cm/y (−1.34 to −1.09 SDS), but by the age of 8.5 years, his height was 119.7 cm (−1.91 SDS) and his growth velocity had slowed to 2.3 cm/year (−4.1 SDS). A glucagon stimulation test performed at the age of 9.8 years (height, 123.8 cm; −2.24 SDS) showed a low peak GH (2.9 μg/liter) with otherwise normal pituitary function. He commenced treatment with recombinant human (rh) GH around the age of 10 years (mean dose, 1 mg/m$^2$/d), progressed normally through puberty, and attained a normal adult height of 170.4 cm (−0.65 SDS (Table 1); midparental height, 169.2 cm, −0.8 SDS) (Figure 2A). Retesting at the end of growth demonstrated persisting GHD with a low IGF1 (6.9 nmol/liter; range, 29.4–117.4), an undetectable peak GH (<0.1 μg/liter) (Table 2) to insulin tolerance test, and otherwise normal pituitary function. A pituitary MRI scan confirmed APH (Figure 2D) and he remained on adult rhGH replacement (0.6 mg/d).

## Patient IV.2

The younger male sibling (Figure 1A) of patient IV.1 first presented at the age of 1.5 years with bilateral undescended testes, micropenis, and a hypoplastic scrotum. He was born at term with a birth weight of 3.64 kg and no history of neonatal problems. At presentation, he had a height of 79.6 cm (−0.5 SDS) with a weight of 9.8 kg (−1.48 SDS) and no

**Table 1.** Auxology on Patients IV.1, IV.2, IV.3, and II.1

| Patient | Sex | Age (y) | Height SDS | Weight SDS | MRI | Tx Age (y) | Tx | Adult Height (cm) | Adult Height SDS |
|---|---|---|---|---|---|---|---|---|---|
| IV.1 | M | 9.8 | −2.24 | −2.15 | APH | 10.3 | rhGH | 170.4 | −0.65 |
| IV.2 | M | 6.2 | −1.23 | −0.48 | APH | 6.5 | rhGH | 173.3 | 1.02 |
| IV.3 | F | 16.0 | −3.0 | −1.06 | APH | Adult | rhGH | 146.3 | −2.7 |
| II.1 | F | 6.0 | −1.8 | −0.34 | APH | 6.0 | rhGH | 166 | 0.66 |

Abbreviations: SDS, standard deviation score; Tx, treatment.

dysmorphic features. A 3-day and 3-week hCG stimulation test showed normal T responses (11.1 and 18.7 nmol/liter, respectively), with baseline gonadotrophins in the prepubertal range (LH < 0.7U/liter, FSH 1.7 U/liter); both testes were visualized in the inguinal canal. By the age of 2 years, he had a further 6-week hCG treatment course with good response in terms of testicular descent. However, at the age of 4 years, he had left testicular torsion with subsequent orchidectomy and right orchidopexy. By the age of 6 years, his height was 110.2 cm (−1.21 SDS) and his growth velocity had slowed to 3.6 cm/year (−2.63 SDS) (Figure 2B). Glucagon stimulation test at that time confirmed GHD with a peak GH of 2.9 μg/liter and a low IGF1 (18 ng/ml; normal range [NR], 45–321 ng/ml) and IGFBP3 (1.24 mg/liter; range, 1.86–4.39) (Table 2) with otherwise normal pituitary function and APH on MRI (Figure 2E). Treatment with rhGH was commenced at the age of 6.5 years with an excellent response. By the age of 14.6 years, he had progressed into puberty with a height of 173.3 cm (±1.02 SDS) and subsequently decided to stop rhGH. He has decided not to attend any further clinics.

### Patient IV.3

The female sibling of patients IV.1 and IV.2 (Figure 1A) first presented at age 16 years with short stature (height, 144 cm; −3.0 SDS). She had already attained menarche and had a clinical phenotype suggestive of untreated GHD

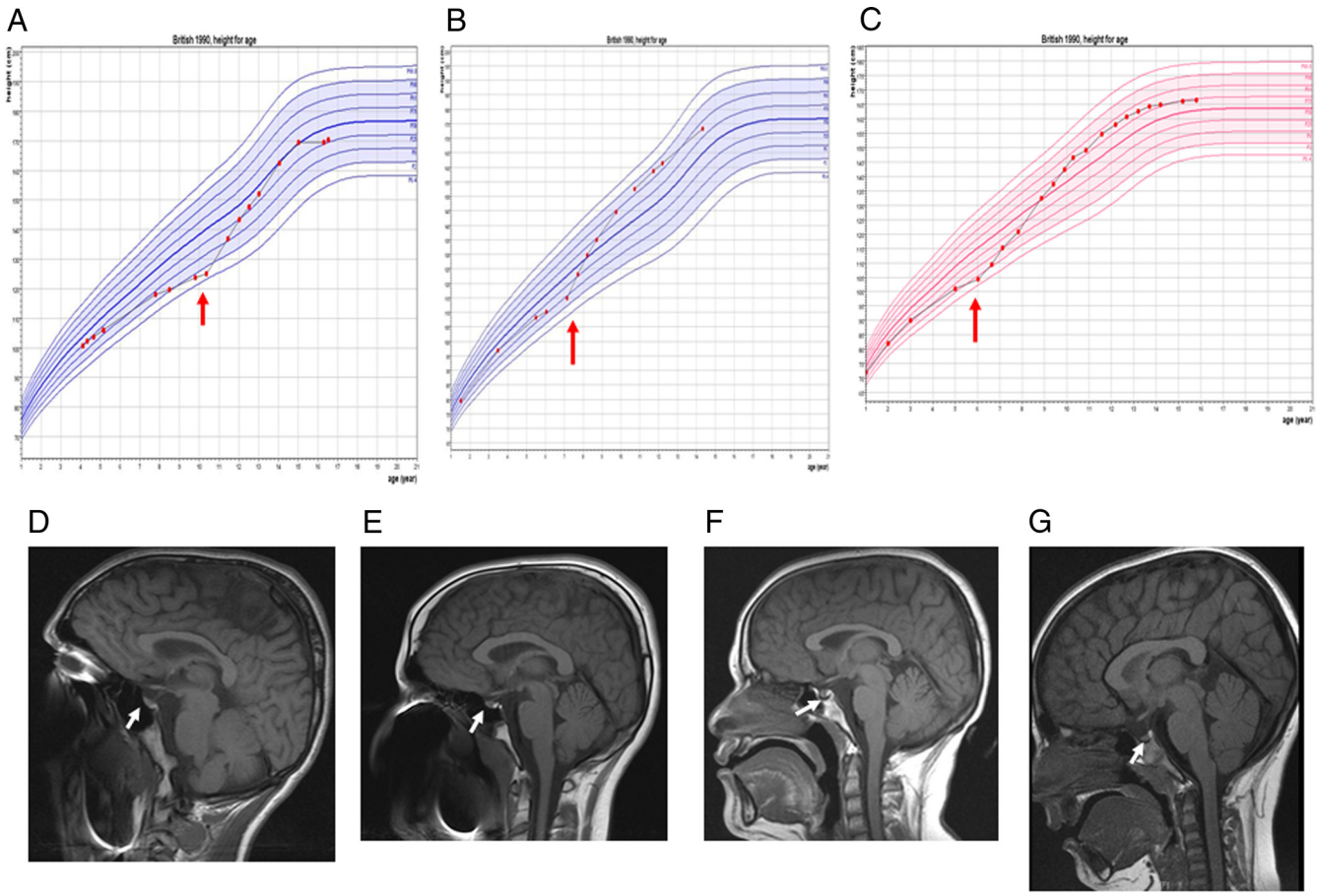


**Figure 2.** A-C, Growth charts of patients IV.1, IV.2, and II.1. A, Growth of patient IV.1 with GH treatment commencing at 10 years of age. B, Growth of patient IV.2 with GH treatment commencing at seven years of age. (C) Growth of patient II.1 with GH treatment commencing at six years of age. D–G, Pituitary MRI scan of patients IV.1, IV.2, IV.3, and II.1, respectively, presenting with a small anterior pituitary (indicated by the arrows).

**Table 2.** Endocrine Testing for Patients IV.1, IV.2, IV.3, and II.1

| Patient | Age y | Peak GH (μg/liter) | IGF-1 (ng/ml) | IGFBP3 (mg/liter) | FT4 (pmol/liter) | TSH (mU/liter) | PRL Peak (mU/liter) | Cortisol (nmol/liter) | E2 (pmol/liter) | LH Basal (IU/liter) | LH Peak (IU/liter) | FSH Basal (IU/liter) | FSH Peak (IU/liter) | T (nmol/liter) | | | Chol (mmol/liter) | Chol/HDL Ratio | US Testes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| IV.1 | 4.2 | — | — | — | — | — | — | — | — | <0.7 | — | 1.0 | — | D0 0.4 | 3 d 4.8 | | — | — | — |
| IV.1 | 9.8 | 2.9 | 18 (NR 64–580) | 1.37 (NR 2.265–5.734) | 17.9 | 1.6 | 249 | 1067 | — | 0.7 | 3.4 | 0.3 | 1.7 | — | | | — | — | — |
| IV.1 | 16.3 | <0.1 | 6.9 (nmol/liter; NR 29.4–117.4) | — | 21.1 | 1.29 | 221 | 688 | — | 4.2 | 11.4 | 3.1 | 4.2 | 32.4 | | | — | — | — |
| IV.2 | 1.6 | — | — | — | — | — | — | — | — | <0.7 | — | 1.7 | — | D0 <0.7 | 3 d 11.1 | 3 wk 18.7 | — | — | Inguinal canal |
| IV.2 | 6.2 | 2.9 | 18 (NR 45–321) | 1.24 (NR 1.86–4.39) | 21.1 | 2.1 | 221 | 669 | — | <0.7 | 10.2 | 5.3 | 18.9 | — | | | — | — | — |
| IV.3 | 16.0 | <0.1 | <3.3 (nmol/liter; NR 30.8–129.5) | 1.15 (NR 3.2–8.7) | 15.8 | 4.63 | 196 | 733 | 123 | 3.4 | — | 5.2 | — | — | | | 4.6 | 3.8 | — |
| II.1 | 6.5 | 1.1 (Glucagon) 1.2 (GHRH) | 17 (NR 45–321) | 1.52 (mg/liter; NR 1.86–4.39) | 19.3 | 2.5 | 71 | 626 | — | — | — | — | — | — | | | — | — | — |

Abbreviations: Chol, cholesterol; FT4, free thyroxine; PRL, prolactin; US, ultrasound.

(abdominal fat deposition, a high-pitched voice, and frontal bossing). She had an undetectable IGF1 (<3.3 nmol/liter), undetectable peak GH to insulin tolerance test (<0.1 μg/liter) (Table 2), a low bone mineral density (−2.5 Z scores in lumbar spine), and APH on MRI (Figure 2F). She was started on adult rhGH replacement (0.6 mg/d) and reached a final height of 146.3 cm (−2.7 SDS) (Table 1). She remains overweight, with acanthosis nigricans suggestive of insulin insensitivity (Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) of 3.1, peak insulin to oral glucose load of 143 mU/liter, with a 2-hour blood glucose level of 5.1 mmol/liter).

### Patient II.1

A female patient (unrelated to pedigree I) born to a consanguineous Pakistani pedigree (Figure 1B) with a birth weight of 3.32 kg, presented at age 6 years with short stature (height, 104.3 cm [−1.8 SDS]; weight, 19.4 kg [−0.34 SDS]), poor growth with a growth velocity of 3.3 cm/year (−3 SDS), and APH on her MRI (Figure 2G). Biochemical testing revealed GH deficiency, with a peak GH to glucagon testing of 1.1 μg/liter, an IGF1 of 17 ng/ml (NR, 45–321 ng/ml), and an IGFBP3 of 1.52 mg/liter (NR, 1.862–4.399 mg/liter) (Table 2). At the age of 6 years, she failed to respond to a GHRH test, and was subsequently started on rhGH treatment at a dose of 0.65 mg/m$^2$/d (Figure 2C). She underwent spontaneous puberty and there were no concerns regarding her physical development. She has achieved a final height of 166 cm (±0.66 SDS) (Table 1). Her father's cousin has two daughters that are on GH treatment for short stature (DNA not available).

### Mutational analysis

Following direct sequencing analysis of three siblings (pedigree I) and an unrelated female patient (pedigree II) with IGHD, two homozygous variants were identified in the *GHRHR* gene in all four patients. The first was a novel homozygous missense variant in exon 1 (*c.11G>A*) (Figure 1Ci) resulting in the substitution of arginine by glutamine (p.R4Q). The second was a novel homozygous missense variant in exon 3 (*c.236C>T*) (Figure 1Cii) resulting in the substitution of proline by leucine (p.P79L). Neither of these changes was identified on control databases including Exome Variant Server, 1000 Genomes, and the ExAC Browser, nor in 200 ethnically matched controls, with the exception of p.R4Q being present once on the ExAC Browser in heterozygous form from a total of 20 396 control alleles. Both p.R4Q and p.P79L have not been previously described and both are located within a highly conserved region between species (Figure 1D). All four patients were also screened for mutations in *GH1* and were negative.

### Protein modeling

Molecular modeling predicts that the GHRHR p.P79L variant will disrupt a disulphide bridge, thus destabilizing the protein. In addition, the protein prediction model Polyphen2 (http://genetics.bwh.harvard.edu/pph2/) predicted p.P79L to be functionally deleterious. Moreover, the crystal structure of a glucagon-like peptide-1 in complex with the extracellular domain of its receptor (likely to have the same structure as the GHRHR extracellular domain) shows that residues close to p.P79 interact with the ligand. Therefore, even if the mutant protein were to fold correctly without the disulphide bond in place (or with a weak disulphide bridge), the mutation is still predicted to disrupt the ligand-binding region.

It was not possible to model the R4Q mutant because the model did not extend far enough into the N-terminus. This region is in the signal peptide and is outside the hydrophobic region shown to be required for function (17). Additionally, the arginine or glutamine at position 4 (p.R4Q) have identical scores for signal peptide prediction (SignalIP4.1).

### Luciferase assays

Functional analysis was performed by monitoring cAMP responses of cells expressing wild-type and mutant GHRHR to varying concentrations of GHRH, and dem-

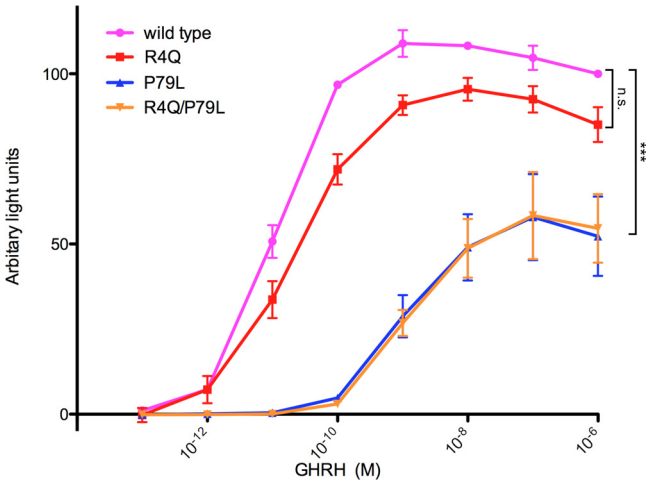


**Figure 3.** Functional analysis of mutant *GHRHR* proteins. Transfection of HEK293 cells with wild-type or mutant GHRHR demonstrating the effects of mutations on GHRHR responses to stimulation with ligand. Transfected cells were stimulated with varying concentrations of GHRH and receptor activation monitored by cAMP accumulation in the cells (evaluated by cotransfection with the cAMP sensor Glosensor). Values shown are the mean ± SE of three independent transfection reactions, with the data normalized to the maximal response of the wild-type receptor for each assay. ***$P < .001$ for both EC50 and maximum cAMP level, n.s.: not significant, one-way ANOVA, with Tukey post hoc test.

onstrated that the double p.R4Q/p.P79L mutation had a significantly reduced maximal activity to 52.0 ± 4.6% of wild-type GHRHR ($P < .001$; Figure 3), with a reduction in affinity for the GHRH1–44 ligand (EC50 p.R4Q/p.P79L $113 \times 10^{-11} \pm 1.51 \times 10^{-11}$ vs WT $1.12 \times 10^{-11} \pm 0.21 \times 10^{-11}$, $P < .001$). Analysis of GHRHR protein with individual mutations demonstrated that p.P79L is responsible for both the reduction in activity (55.3 ± 4.4% of wild-type, $P < .001$) and the altered affinity (EC50 $113 \times 10^{-11} \pm 1.51 \times 10^{-11}$, $P < .001$ vs WT; nonsignificant difference vs p.R4Q/pP79L) (Figure 3). The single p.R4Q mutation had no significant effect on either maximal activity ($P = .65$) or EC50 ($P = .9$) compared with wild-type GHRHR (Figure 3). Western analysis of cell extracts demonstrated no significant difference in the expression levels of the various forms of GHRHR (data not shown).

## Discussion

We report two novel homozygous *GHRHR* variants in three siblings (pedigree I; IV.1-IV.3) and in an unrelated patient (pedigree II; II.1), from consanguineous families from the South East Asian community, suggesting a possible founder effect. Pedigree I (incorporating patients IV.1–IV.3) is multiply consanguineous, with the parents of the probands being first cousins. Despite all patients having IGHD and APH on their MRI scans, the combined effect of these variants is variable in terms of height deficit, and the patients' phenotypes are mild compared to previous reports, with presentation in mid-childhood. Indeed, the untreated female patient from pedigree I presented much later, after she had almost completed her growth, and reached a near-adult height of 144 cm (−3.0 SDS). Compared to the mean of approximately 114 cm in the literature, this is the tallest untreated height reported for a patient with a *GHRHR* mutation to our knowledge. Subsequent treatment with an adult replacement dose of rhGH resulted in an improvement in her final height to 146.3 cm (−2.7 SDS). Surprisingly, the clinical presentation of the two brothers within the same pedigree with bilateral undescended testes, hypoplastic scrotum, and micropenis was suggestive of hypogonadotrophic hypogonadism, although endocrine testing confirmed that the gonadal axis was intact; the brothers progressed normally through puberty, with normalization of phallic size after commencement of rhGH treatment. The older brother and sister are now treated with adult GH replacement therapy.

The asymptomatic mother of patients IV.1 through IV.3 was a heterozygous carrier of both variants, and the father is also expected to be a carrier, although his DNA is unavailable. The presence of these two homozygous variants in the two ostensibly unrelated families raises the possibility that pedigrees I and II are distantly related or may originate from the same area in South East Asia.

Apart from a single report (18), patients with *GHRHR* mutations do not have neonatal hypoglycemia and in all reports to date they are reported to have normal genitalia. This is the first report of male patients with *GHRHR* mutations presenting with a micropenis and bilateral undescended testes. The mechanism underlying this presentation is unknown.

Several previously reported missense *GHRHR mutations* (p.H137L, p.L144H, p.A176V, p.A222E, p.F242C, p.K329E) have been shown to result in correct surface expression of the receptor but reduced ability to bind to GHRH, thereby impairing intracellular signaling and stimulation of GH secretion (19, 13, 20). However, a missense mutation (p.V10G) within the signal peptide has been shown to affect the correct processing of the receptor and results in incomplete cleavage of the signal peptide with failure of the mutant GHRHR receptor to translocate to the cell surface (17). The first variant, p.R4Q in exon 1, results in the substitution of a strongly basic arginine residue by a neutral glutamine residue. Despite our p.R4Q variant being located in the signal peptide, when arginine is substituted by tryptophan (p.R4W) at position 4, there is unaltered function; this is consistent with our functional

data whereby the p.R4Q variant appears to retain function (17).

The second variant, p.P79L in exon 3, results in the substitution of a proline residue by leucine. Proline is known to be essential for protein folding (21); therefore, its loss at this highly conserved position will likely affect protein conformation, which supports our protein prediction model for p.P79L. The functional assays performed further support this and conclude that the p.P79L mutation alters the binding affinity and activity of GHRHR, and is thus the likely cause of the GHD observed in patients IV.1-IV.3 and II.1. Therefore the 50% reduction in the maximal cAMP response to stimulation with GHRH observed by the p.R4Q/p.P79L double mutant receptor is most likely because of this pathogenic p.P79L mutation alone rather than the combination of both p.R4Q and p.P79L (Figure 3). Our studies do not rule out the possibility that the p.R4Q variant may be contributory in some way to the mild phenotype.

## Conclusion

We report the presence of two novel homozygous variants in *GHRHR* in a pedigree, and an unrelated patient with IGHD, suggesting a possible founder effect of these variants in patients with IGHD originating from a certain area of South East Asia. The initial phenotype of all patients appears to be relatively mild, despite the presence of the two variants in the same gene. We show here the importance of performing functional studies in this highly unusual scenario where two variants are present in compound homozygosity in affected individuals. All previously reported *GHRHR* mutations have been associated with complete loss of function. Our functional studies have shown that the novel p.P79L variant is pathogenic with what appears to be a partial loss of function, and is most likely the cause of the unusually mild form of IGHD in all four patients. Additionally, the female sibling in pedigree I has the tallest recorded height for an untreated patient with a *GHRHR* mutation; our data therefore suggest the possibility that rare patients with "idiopathic" short stature may manifest mild genetic forms of GHD and reach the target height range for the family without treatment.

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