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1 **Partial loss of function of the GHRH Receptor leads to mild**
2 **Growth Hormone Deficiency**

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14

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52 **Abstract**

53 **Introduction** Recessive mutations in *GHRHR* are associated with severe isolated GH
54 deficiency (IGHD), with a final height in untreated patients of 130cm±10cm (-
55 7.2±1.6SDS; males) and 114±0.7cm (-8.3±0.1SDS; females). **Objective** We
56 hypothesised that a consanguineous Pakistani family with IGHD in 3 siblings (2
57 males, 1 female) would have mutations in *GHI* or *GHRHR*. **Results** Two novel
58 homozygous missense variants [*c.11G>A* (p.R4Q), *c.236C>T* (p.P79L)] at conserved
59 residues were identified in all 3 siblings. Both were absent from control databases,
60 aside from pR4Q appearing once in heterozygous form in the ExAc Browser. The
61 brothers were diagnosed with GHD at 9.8 and 6.0 years (height SDS: -2.24 and -1.23
62 respectively), with a peak GH of 2.9 µg/l with low IGF-1/IGFBP3. Their sister
63 presented at 16 years with classic GHD (peak GH <0.1µg/l, IGF-1<3.3mmol/L) and
64 attained an untreated **near-adult height** of 144 cm (-3.0 SDS); the tallest untreated
65 patient with *GHRHR* mutations reported. An unrelated Pakistani female IGHD patient
66 was also compound homozygous. All patients had a small anterior pituitary on MRI.
67 Functional analysis revealed a 50% reduction in maximal cAMP response to
68 stimulation with GHRH by the p.R4Q/p.P79L double mutant receptor, with a 100 fold
69 increase in EC50. **Conclusion** We report the first co-existence of two novel
70 compound homozygous *GHRHR* variants in 2 unrelated pedigrees associated with a
71 partial loss of function. Surprisingly, the patients have a relatively mild IGHD
72 phenotype. Analysis revealed that the pP79L mutation is associated with the
73 compromise in function, with the residual partial activity explaining the mild
74 phenotype.

75

76

77 **Introduction**

78 The gene encoding the growth hormone releasing hormone receptor (*GHRHR*)
79 is 15.51kb in length and incorporates 13 exons on chromosome 7p14. It encodes a G-
80 protein coupled receptor (423aa) and is expressed on the somatotroph cells of the
81 anterior pituitary (1). Its ligand growth hormone releasing hormone (GHRH), released
82 from the hypothalamus, stimulates the synthesis and release of growth hormone (GH;
83 encoded by *GHI*) upon binding in the presence of the pituitary-specific transcription
84 factor POU1F1 (PIT1) (2,3). GH in turn binds to receptors on the liver and generates
85 insulin-like growth factor 1 (IGF1) and insulin-like growth factor binding protein 3
86 (IGFBP3), thereby promoting growth.

87 Consistent with their role in growth regulation, mutations in *GHRHR*, *GHI*
88 and *SOX3* are implicated in the etiology of isolated growth hormone deficiency
89 (IGHD) (4), and the pathway was recently implicated in the GHD phenotype observed
90 in the autosomal dominant disorder pseudohypoparathyroidism type 1b (5).
91 Autosomal recessive mutations occurring in the *GHRHR* gene have been implicated
92 in severe IGHD Type 1B, also known as Sindh dwarfism (6,7). Reported aberrations
93 in *GHRHR* have included missense, splice (8), nonsense (9,10), microdeletion and
94 promoter mutations (11,12). Many have been shown to specifically affect cAMP
95 production, for example *GHRHR* (p.K329E), which fails to show a cAMP response
96 after treatment with GHRH (13). All mutations described to date have shown a
97 complete loss of function.

98 Severe IGHD Type 1B was initially described in pedigrees from the Indian
99 subcontinent (14) and Brazil (15). Interestingly the phenotype is usually not that of
100 classic IGHD in that affected patients have minimal facial hypoplasia and no
101 microphallus, but do manifest anterior pituitary hypoplasia (APH) on their magnetic

102 resonance imaging (MRI) (3). However, growth failure is severe with proportionate
103 dwarfism and pubertal delay, and biochemically, the patients have low GH and IGF1
104 concentrations with otherwise normal pituitary function. To date, reported height in
105 untreated patients with a *GHRHR* mutation is on average $130 \pm 10\text{cm}$ ($-7.2 \pm 1.6\text{SDS}$)
106 in males and $114 \pm 0.7\text{cm}$ ($-8.3 \pm 0.1\text{SDS}$) in females (16).

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

116

117 **Materials and Methods**

118

119 **Patients**

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

124

125 **Direct Sequencing Analysis**

126 Three siblings with IGHD from Pedigree 1 and a separate patient from
127 Pedigree 2 were screened for *GHI* and *GHRHR* mutations. The coding region of these

128 genes consists of 5 exons in *GHI* and 13 exons in *GHRHR*. These were amplified by
129 PCR on an Eppendorf Thermocycler over 35 cycles with primers designed using the
130 Primer3 program (available at <http://frodo.wi.mit.edu/primer3>) flanking each of the
131 exons in the coding regions of the genes. PCR products were treated with MicroClean
132 reagent (Web Scientific, cat # 2MCL-10) according to manufacturer's instructions
133 and then sequenced using BigDye v1.1 sequencing chemistry (Applied Biosystems)
134 and analysed on a 3730X1 DNA Analyzer (Applied Biosystems/Hitachi, Japan, cat #
135 625-0020). Details of the PCR conditions are available upon request including the
136 primer sequences, product sizes and annealing temperatures. For any mutations
137 identified, control databases were consulted as follows: Exome Variant Server
138 (evs.gs.washington.edu/EVS/) (EVS), 1000 Genomes (www.1000genomes.org), an
139 in-house panel of 200 ethnically matched controls, and the Exome Aggregation
140 Consortium (ExAC Browser) (<http://exac.broadinstitute.org/>).

141 **Molecular modelling**

142 The RasMol prediction model database was used to build a 3D annotated
143 model of the GHRHR wild type and mutant proteins respectively, to analyse and
144 compare protein folding and structure.

145 **Functional analysis:**

146 An expression vector was obtained encoding full-length wild-type *GHRHR*
147 cloned into pcDNA3.1 (Source Bioscience). Detected mutations p.R4Q, p.P79L and
148 the double mutant p.R4Q/p.P79L were introduced into the sequence using the
149 QuikChange II XL Site-Directed Mutagenesis Kit (Agilent Technologies UK LTD).
150 Vectors were transfected into HEK293 cells (American Type Culture Collection)
151 cultured in DMEM supplemented with 10% foetal bovine serum, 100U/ml penicillin,

152 100µg/ml streptomycin and 1% non-essential amino acids at 37°C in a humidified 5%
153 CO₂ incubator. Approximately 1x10⁶ cells were transfected with 1.2 µg Glosensor
154 22F (Promega, Madison, WI, USA) and 1.2 µg GHRHR using Polyjet transfection
155 reagent (SignaGen laboratories, Gaithersburg, MD, USA) according to the
156 manufacturer's instructions. Cells were plated in a white 96-well dish at a density of
157 approximately 35,000 cells per well and the following day media replaced with
158 Leibovitz's L-15 medium (Thermo Fisher Scientific, Waltham, MA, USA) containing
159 2mM luciferin (Promega). After equilibration at 25°C, the basal luciferase activity
160 was measured on a Glomax luminometer (Promega) and cells were then stimulated
161 with various concentrations of GHRH 1-44 (Bachem, Bubendorf, Switzerland) and
162 the luciferase response monitored approximately every 3 minutes over a period of at
163 least 60 minutes. Response to GHRH was calculated as the area under the curve for
164 the time period of measurement after correction for background activity from
165 unstimulated cells.

166

167 **Results**

168 **Patient phenotypes:**

169 ***Patient IV.1***

170 The proband was a male born at term (birth weight 3.6 kg) to a
171 consanguineous Pakistani family (Figure 1A), and first presented at the age of 4 years
172 with bilateral undescended testes, micropenis and a hypoplastic scrotum. There was
173 no history of neonatal hypoglycemia or jaundice, he had no dysmorphic features, and
174 at presentation his height was 100.7cm (-0.73 SDS) with a weight of 14.8kg (-1.09
175 SDS). At the age of 4.2 years he had an acceptable testosterone response to a 3-day
176 human chorionic gonadotrophin (hCG) stimulation test, rising from 0.4 to 4.8nmol/L

177 and basal gonadotrophins in the pre-pubertal range (LH <0.7 U/L, FSH 1.0 U/L).
178 Following hCG stimulation, testes were bilaterally palpable; however, later
179 examination revealed impalpable testes and he received a further 6-week course of
180 treatment with hCG at the age of 7 years with a partial response, and underwent
181 bilateral orchidopexies at the age of 8.2 years. Between the ages of 4-7 years, he grew
182 steadily with a growth velocity of 5.0-5.5 cm/year (-1.34 to -1.09 SDS), but by the age
183 of 8.5 years, his height was 119.7cm (-1.91 SDS) and his growth velocity had slowed
184 to 2.3 cm/year (-4.1 SDS). A glucagon stimulation test performed at the age of 9.8
185 years (Ht 123.8cm, -2.24 SDS) showed a low peak GH (2.9µg/L) with otherwise
186 normal pituitary function. He commenced treatment with recombinant human (rh) GH
187 around the age of 10 years (mean dose 1mg/m²/day), progressed normally through
188 puberty and attained a normal adult height of 170.4 cm (-0.65 SDS (Table 1); mid-
189 parental height of 169.2 cm, -0.8 SDS) (Figure 2A). Retesting at the end of growth
190 demonstrated persisting GHD with a low IGF1 (6.9 nmol/l; range 29.4-117.4), an
191 undetectable peak GH (<0.1 µg/L) (Table 2) to insulin tolerance test, and otherwise
192 normal pituitary function. A pituitary MRI confirmed APH (Figure 2D) and he
193 remained on adult rhGH replacement (0.6mg/day).

194

195 ***Patient IV.2***

196 The younger male sibling (Figure 1A) of patient IV.1 first presented at the age
197 of 1.5 years with bilateral undescended testes, micropenis and a hypoplastic scrotum.
198 He was born at term with a birth weight of 3.64 kg and there was no history of
199 neonatal problems. At presentation he had a height of 79.6 cm (-0.5 SDS) with a
200 weight of 9.8 kg (-1.48 SDS) and no dysmorphic features. A 3-day and 3-week HCG
201 stimulation test showed normal testosterone responses (11.1 nmol/l and 18.7 nmol/l

202 respectively), with baseline gonadotrophins in the prepubertal range (LH <0.7U/L,
203 FSH 1.7U/L); both testes were visualised in the inguinal canal. By the age of 2 years,
204 he had a further 6-week hCG treatment course with good response in terms of
205 testicular descent. However, at the age of 4 years, he had left testicular torsion with
206 subsequent orchidectomy and right orchidopexy. By the age of 6 years his height was
207 110.2 cm (-1.21 SDS) and his growth velocity had slowed to 3.6cm/year (-2.63 SDS)
208 (Figure 2B). Glucagon stimulation test at that time confirmed GHD with a peak GH
209 of 2.9µg/l and a low IGF1 (18 ng/ml; normal range (NR) 45-321 ng/ml) and IGFBP3
210 (1.24 mg/l; range 1.86-4.39) (Table 2) with otherwise normal pituitary function and
211 APH on MRI (Figure 2E). Treatment with rhGH was commenced at the age of 6.5
212 years with an excellent response. By the age of 14.6 years, he had progressed into
213 puberty with a height of 173.3cm (+1.02 SDS) and subsequently decided to stop
214 rhGH. He has decided not to attend any further clinics.

215

216 ***Patient IV.3***

217 The female sibling of patients IV.1 and IV.2 (Figure 1A) first presented at age
218 16 years with short stature (height 144cm, -3.0 SDS). She had already attained
219 menarche and had a clinical phenotype suggestive of untreated GHD (abdominal fat
220 deposition, a high pitched voice and frontal bossing). She had an undetectable IGF1
221 (<3.3 nmol/L), undetectable peak GH to insulin tolerance test (<0.1 µg/l) (Table 2), a
222 low bone mineral density (-2.5 Z scores in lumbar spine) and APH on MRI (Figure
223 2F). She was commenced on adult rhGH replacement (0.6mg/day) and reached a final
224 height of 146.3cm (-2.7 SDS) (Table 1). She remains overweight, with acanthosis
225 nigricans suggestive of insulin insensitivity (HOMA-IR of 3.1, peak insulin to oral
226 glucose load of 143 mU/L, with a 2 hour blood glucose of 5.1 mmol/L).

227

228

229

230 ***Patient II.1***

231 A female patient (unrelated to pedigree I) born to a consanguineous Pakistani
232 pedigree (Figure 1B) with a birth weight of 3.32 kg, presented at age 6 years with
233 short stature [height 104.3 cm (-1.8 SDS), weight 19.4 kg (-0.34 SDS)], poor growth
234 with a growth velocity of 3.3 cm/year (-3 SDS), and APH on her MRI (Figure 2G).
235 Biochemical testing revealed GH deficiency, with a peak GH to glucagon testing of
236 1.1µg/l, an IGF1 of 17 ng/ml (NR 45-321 ng/ml) and an IGFBP3 of 1.52 mg/L (NR
237 1.862-4.399 mg/L) (Table 2). At the age of 6 years she failed to respond to a GHRH
238 test, and was subsequently commenced on rhGH treatment at a dose of
239 0.65mg/m²/day (Figure 2C). She underwent spontaneous puberty and there were no
240 concerns regarding her physical development. She has achieved a final height of 166
241 cm (+0.66 SDS) (Table 1). Her father's cousin has two daughters that are on GH
242 treatment for short stature (DNA not available).

243

244 **Mutational analysis**

245 Following direct sequencing analysis of three siblings (pedigree I) and an
246 unrelated female patient (pedigree II) with IGHD, two homozygous variants were
247 identified in the *GHRHR* gene in all four patients. The first was a novel homozygous
248 missense variant in exon 1 (*c.11G>A*) (Figure 1Ci) resulting in the substitution of
249 arginine by glutamine (p.R4Q). The second was a novel homozygous missense
250 variant in exon 3 (*c.236C>T*) (Figure 1Cii) resulting in the substitution of proline by
251 leucine (p.P79L). Neither of these changes were identified on control databases

252 including Exome Variant Server, 1000 genomes and the ExAc Browser, nor in 200
253 ethnically-matched controls, with the exception of p.R4Q being present once on the
254 ExAc browser in heterozygous form out of a total of 20,396 control alleles. Both
255 p.R4Q and p.P79L have not been previously described and both are located within a
256 highly conserved region between species (Figure 1D). All four patients were also
257 screened for mutations in *GHI* and were negative.

258

259 **Protein modelling**

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

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

274

275 **Luciferase assays**

276 Functional analysis was performed by monitoring cAMP responses of cells
277 expressing wild-type and mutant GHRHR to varying concentrations of GHRH, and
278 demonstrated that the double p.R4Q/p.P79L mutation had a significantly reduced
279 maximal activity to 52.0+/-4.6% of wild-type GHRHR (p<0.001; Figure 3), with a
280 reduction in affinity for the GHRH1-44 ligand (EC50 p.R4Q/p.P79L 113×10^{-11} +/-
281 1.51×10^{-11} vs WT 1.12×10^{-11} +/- 0.21×10^{-11} , p<0.001). Analysis of GHRHR protein
282 with individual mutations demonstrated that p.P79L is responsible for both the
283 reduction in activity (55.3+/-4.4% of wild-type, p<0.001) and the altered affinity
284 (EC50 113×10^{-11} +/- 1.51×10^{-11} , p<0.001 vs WT; non-significant difference vs
285 p.R4Q/p.P79L) (Figure 3). The single p.R4Q mutation had no significant effect on
286 either maximal activity (p = 0.65) or EC50 (p = 0.9) compared with wild type
287 GHRHR (Figure 3). Western analysis of cell extracts demonstrated no significant
288 difference in the expression levels of the various forms of GHRHR (data not shown).

289

290 **Discussion**

291 We report two novel homozygous *GHRHR* variants in three siblings (Pedigree
292 I; IV.1-IV.3), and in an unrelated patient (Pedigree 2; II.1), from consanguineous
293 families from the South East Asian community, suggesting a possible founder effect.
294 Pedigree I (incorporating patients IV.1-IV.3) is multiply consanguineous, with the
295 parents of the probands being first cousins. Despite all patients having IGHD and
296 APH on their MRI, the combined effect of these variants is variable in terms of height
297 deficit, and the patients' phenotypes are mild compared to previous reports, with
298 presentation in mid-childhood. Indeed, the untreated female patient from pedigree I
299 presented much later, after she had almost completed her growth, and reached a **near-**
300 **adult height** of 144cm (-3.0 SDS). Compared to the mean of ~114cm in the literature,

301 this is the tallest untreated height reported for a patient with a *GHRHR* mutation to
302 our knowledge. Subsequent treatment with an adult replacement dose of rhGH
303 resulted in an improvement in her final height to 146.3 cm (-2.7 SDS). Surprisingly,
304 the clinical presentation of the two brothers within the same pedigree with bilateral
305 undescended testes, hypoplastic scrotum and micropenis was suggestive of
306 hypogonadotrophic hypogonadism, although endocrine testing confirmed that the
307 gonadal axis was intact and they progressed normally through puberty, with
308 normalization of phallic size after commencement of rhGH treatment. The older
309 brother and sister are now treated with adult GH replacement therapy.

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

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

320 A number of previously reported missense *GHRHR* mutations (p.H137L,
321 p.L144H, p.A176V, p.A222E, p.F242C, p.K329E) have been shown to result in
322 correct surface expression of the receptor but reduced ability to bind to GHRH,
323 thereby impairing intracellular signalling and stimulation of GH secretion (19,13,20).
324 However, a missense mutation (p.V10G) within the signal peptide has been shown to
325 affect the correct processing of the receptor and results in incomplete cleavage of the

326 signal peptide with failure of the mutant GHRHR receptor to translocate to the cell
327 surface (17). The first variant, p.R4Q in exon 1, results in the substitution of a
328 strongly basic arginine residue by a neutral glutamine residue. Despite our p.R4Q
329 variant being located in the signal peptide, when arginine is substituted by tryptophan
330 (p.R4W) at position 4 there is unaltered function, and this is consistent with our
331 functional data whereby the p.R4Q variant appears to retain function (17).

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

343

344 **Conclusion**

345 We report the presence of two novel homozygous variants in *GHRHR* in a
346 pedigree, and an unrelated patient with IGHD, suggesting a possible founder effect of
347 these variants in patients with IGHD originating from a certain area of South-East
348 Asia. The initial phenotype of all patients appears to be relatively mild, despite the
349 presence of the two variants in the same gene. We show here the importance of
350 performing functional studies in this highly unusual scenario where two variants are

351 present in compound homozygosity in affected individuals. All previously reported
352 *GHRHR* mutations have been associated with complete loss of function. Our
353 functional studies have shown that the novel p.P79L variant is pathogenic with what
354 appears to be a partial loss of function, and is most likely the cause of the unusually
355 mild form of IGHD in all four patients. Additionally, the female sibling in pedigree 1
356 has the tallest recorded height for an untreated patient with a *GHRHR* mutation, and
357 our data therefore suggest the possibility that rare patients with “idiopathic” short
358 stature may manifest mild genetic forms of GHD and reach the target height range for
359 the family without treatment.

360

361

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441

442 **Figures**

443 **Figure 1. (A) Consanguineous Pakistani pedigree with IGHD.** This family tree
444 shows two male probands in Pedigree 1 and their affected sister (shaded black squares
445 and a shaded circle respectively). The double lines represent consanguinity, with the
446 parents of the affected patients being first cousins. The generations within the family
447 are indicated by roman numerals. **(B) Pedigree II with IGHD.** This family consists
448 of one affected female (shaded black circle) and her unaffected sister, born to first

449 cousin parents. **(C) Two *GHRHR* mutations associated with IGHD phenotypes.** A
450 novel homozygous missense mutation, c.11G>A causing a p.R4Q substitution, was
451 identified in exon 1 ('(i)' - shown as 'N' and indicated by arrow) and a homozygous
452 missense mutation; c.236C>T, causing a p.P79L substitution, was found in exon 3
453 ((ii)' – shown by 'N' and indicated by arrow) in three siblings from pedigree I and in
454 an unrelated female patient from pedigree II. **(D) Highly conserved residues across**
455 **multiple species.** *GHRHR* protein sequences spanning both amino acids that are
456 substituted in the patients. The p.R4 and p.P79 are represented in green and show high
457 conservation between multiple species. Any spanning amino acid residues that differ
458 from the reference human sequence are highlighted in red.

459

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

466

467 **Figure 3. Functional analysis of mutant *GHRHR* proteins.** Transfection of HEK293
468 cells with wild-type or mutant *GHRHR* demonstrating the effects of mutations on
469 *GHRHR* responses to stimulation with ligand. Transfected cells were stimulated with
470 varying concentrations of GHRH and receptor activation monitored by cAMP
471 accumulation in the cells (evaluated by cotransfection with the cAMP sensor Glosensor).
472 Values shown are the mean+/- SE of three independent transfection reactions, with the
473 data normalised to the maximal response of the wild-type receptor for each assay. ***:

474 $p < 0.001$ for both EC50 and maximum cAMP level, n.s.: not significant, one-way
475 ANOVA, with Tukey post-hoc test.

476

477 **Table 1. Auxological parameters of affected patients.**

478

479 **Table 2. Endocrine data from Pedigrees I and II.** Endocrine values relative to age
480 and MRI results for all patients: IV.1, IV.2, IV.3 and II.1.

Table 1: Auxology on patients IV.1, IV.2, IV.3 and II.1

Patient	Sex	Age (yrs)	Ht SDS	Wt SDS	MRI	Tx Age (yrs)	Tx	Adult Ht (cm)	Adult Ht 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

Table 2: Endocrine testing for patients IV.1, IV.2, IV.3 and II.1

Patient	Age yrs	Peak GH ($\mu\text{g/L}$)	IGF-1 (ng/ml)	IGFBP3 (mg/L)	FT4 (pmol/L)	TSH (mU/L)	PRL (mU/L)	Cortisol peak (nmol/L)	E2 (pmol/L)	LH Basal (IU/L)	LH Peak (IU/L)	FSH Basal (IU/L)	FSH Peak (IU/L)	Testo (nmol/L)			Chol (mmol/L)	Chol /HDL ratio	US testes
														D0	3d	3wk			
IV.1	4.2	-	-	-	-	-	-	-	-	<0.7	-	1.0	-	D0 0.4	3d 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/L; 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	3d 11.1	3wk 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/L; 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/L; NR 1.86- 4.39)	19.3	2.5	71	626	-	-	-	-	-				-	-	-





