Evolution of gonadotropin deficiency in a patient with type II autosomal dominant GH deficiency

James P G Turton, Charles R Buchanan1, Iain C A F Robinson1, Simon J B Aylwin2 and Mehul T Dattani
Biochemistry, Endocrinology and Metabolism Unit and London Centre for Paediatric Endocrinology, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK, 1Departments of Child Health and 2Endocrinology, Kings College Hospital, London, UK and 3Division of Molecular Neuroendocrinology, National Institute for Medical Research, Mill Hill, London, UK
(Correspondence should be addressed to M T Dattani; Email: mdattani@ich.ucl.ac.uk)

Abstract
Background: Type II isolated GH deficiency (IGHD type II) is caused by dominant negative splicing or point mutations of the GH-1 gene. Studies have suggested that dominant mutant GH forms prevent the secretion of wild-type GH, resulting in eventual cell death; surprisingly, some patients with these GH mutations develop other hormonal deficiencies (ACTH, TSH).
Subjects: The proband presented at the age of 2.3 years with IGHD. His father, also known to have been treated for IGHD as a child, had subsequently been lost to follow-up, having remained without treatment during this time. At re-evaluation at the age of 38 years, he complained of lack of stamina and poor libido. Clinical and biochemical assessment confirmed severe GHD, borderline ACTH insufficiency, suboptimal basal and stimulated gonadotropins, and a poor prolactin response to provocation. The basal testosterone concentration was low, and he complained of secondary infertility. Magnetic resonance imaging revealed anterior pituitary hypoplasia in both patients. Genetic testing revealed a heterozygous splicing mutation in GH-1 (intervening sequence-3G→A) in both patients, known to cause IGHD type II.
Interventions: The proband showed an excellent growth response to recombinant human GH (rhGH). His father, also treated with rhGH, showed improved quality of life on rhGH, but testosterone concentrations continued to decline, necessitating treatment with testosterone with symptomatic benefit but no improvement in semen quality.
Conclusions: This case supports recent experimental and clinical observations suggesting that the cytotoxicity associated with accumulation of dominant negative mutant 17.5 kDa GH causes a form of GHD that can evolve into multiple hormone deficiencies. Hence, patients diagnosed initially with IGHD type II require continued long-term clinical follow-up.

Introduction
Four distinct familial forms of isolated growth hormone deficiency (IGHD) have been described (1, 2). Large homozygous deletions (6.7, 7.0, 7.6, and 45 kb) within the GH-1 gene associated with the complete absence of growth hormone (GH) lead to GHD type IA, and GH treatment is frequently associated with the development of antibodies. Additionally, frameshift and nonsense mutations may also be associated with type IA GHD. Type IB GHD is associated with less severe short stature and detectable concentrations of GH, and is due to recessive mutations within GH-1 and GHRHR. Type II autosomal dominant GHD is associated with heterozygous splice site and occasional mis-sense mutations (R183H, V110F, and P89L) within the GH-1 gene (1), while type III GHD is inherited as an X-linked condition. Recent studies have revealed novel insights into the pathogenesis of autosomal dominant IGHD type II. The condition is classically associated with abnormal splicing within GH-1 and is most frequently due to mutations within the first 6 bp of intervening sequence 3 (5’IVS-3) (3). The GH-1 gene is located on the long arm of chromosome 17 (17q22) and consists of five exons encoding human GH, a 191 amino acid (22 kDa) peptide, accounting for more than 80% of circulating GH, with approximately 5–10% consisting of a 20 kDa form of human GH (hGH) that arises as a result of the use of a cryptic 3’ splice site in exon 3. Mutations at the donor splice site of IVS-3 have been shown to generate alternatively spliced transcripts lacking sequences corresponding to exon 3 (Fig. 1). When translated, these produce a 17.5 kDa variant of hGH, which lacks aa 32–71, the entire loop that connects helices 1 and 2 in the tertiary structure of hGH (4–6).

A number of other mutations are also associated with aberrant splicing, and result in the clinical phenotype of...
IGHD type II, including mutations in an exon splice enhancer (ESE) (7–10) and an intron splice enhancer (ISE) (11, 12). These mutations are associated with variably increased levels of exon 3-skipped transcripts (7, 9, 12). The length of intron 3 is also critical in determining the splicing characteristics of the GH-1 gene (10).

Following these observations, additional hormone (partial thyroid-stimulating hormone (TSH) and/or adrenocorticotropic hormone (ACTH)) deficiencies were detected in 8 of 57 patients with IGHD type II (13) due to the IVS-3C1(nZ5) and P89L(nZ3) mutations. Of these, two patients had overt hypothyroidism and ACTH insufficiency as measured by low thyroxine and fasting morning cortisol concentrations, whereas the remainder had ACTH insufficiency. The hypothalamo–pituitary–gonadal endocrine axis was not investigated in these patients, but the authors reported that the gonadotrope axis appeared to be clinically normal.

At a functional level, the 17.5 kDa variant generated by exon 3 skipping has been shown to disrupt the Golgi apparatus and impair trafficking of GH (14). In secretory, but not in non-secretory, cell lines co-expression of Δexon3hGH inhibited wild-type (WT)-hGH secretion in a concentration-dependent manner (15). The aberrant hGH, therefore, acts in a dominant negative manner in the presence of WT-GH (9, 15–17). In a transgenic animal model of IGHD type II, mice expressing Δexon3hGH showed a phenotype characterized by dwarfism, reduced GH content in the anterior pituitary, marked anterior pituitary hypoplasia, and, in the most severely affected lines, the contents of other pituitary hormones (prolactin, TSH, and luteinizing hormone (LH)) were all significantly reduced in adult males (17).

We now report a pedigree in which a father and son presented with short stature due to GHD. The father had been treated with both pituitary-derived and recombinant hGH in childhood, but had been untreated for 20 years. Following the diagnosis of GHD in his son, the father was re-evaluated and reported a lack of energy and vitality and later complained of secondary subfertility. Investigations not only confirmed GHD, but also revealed partial ACTH, prolactin, and gonadotropin deficiencies. Thus, we now show that gonadotropin insufficiency is also associated with this GH-1 mutation in human IGHD type II.

**Methods**

**Clinical evaluation**

Pituitary function was assessed using standard dynamic tests (18). Magnetic resonance imaging (MRI) (1.5 T Siemens Magnetom Symphony, Siemens Medical Systems, Malvern, PA, USA) included T1 and T2 weighted high-resolution pituitary imaging through the hypothalamo–pituitary axis (T1 sagittal 3 mm slices, T1 and T2 coronal 3 mm slices).

**Assays**

GH was measured using the Nichols Advantage method standardized against the International Standard 80/505. Insulin-like growth factor I (IGF-I) and IGF-binding protein-3 (IGFBP-3) were measured using the Nichols Advantage 2-site chemiluminescent assay. TSH, free thyroxine (fT4), prolactin, LH, follicle-stimulating hormone (FSH), cortisol, and testosterone measurements were all performed on the chemiluminescent Bayer ADVIA-Centaur analyzer.

**Genetic investigation**

Following Institutional Review Board Approval and with informed consent, GH-1 was amplified using primers (available on request) flanking exons and including splice sites. IVS-3 was included in overlapping amplicons of exons 3 and 4 and all primers were designed with the aid of WAVEMAKER software (Transgenomic Ltd, Elancourt, France). PCR products were sequenced using the BigDye Terminator Thermal Cycle Sequencing protocol and run out on the ABI 377 Genetic Analyzer (both Applied Biosystems, Cheshire, UK) in both the forward and the reverse orientations and analyzed using Sequencher (Gene Codes Corporation, MI, USA).
Results

Phenotype

The proband (III.2, Fig. 2A), presented at the age of 2.3 years with short stature (height 75 cm (−3.4 SDS), weight 9.35 kg (−2.5 SDS), mid-parental height 164 cm (−1.6 SDS)). His birth weight was 3.35 kg (−0.5 SDS) with a birth length of 52 cm (0.70 SDS). His height velocity had been suboptimal since infancy (Fig. 2B), and his dentition was delayed. On presentation, his facial features were suggestive of a diagnosis of GHD (Fig. 2C); his voice was high-pitched, and his phallus small, with both testes in the scrotum. Glucagon provocation revealed profound GHD (peak GH concentration 2.7 mU/l, IGF-I < 2 nmol/l), with normal cortisol secretion (peak cortisol concentration 916 nmol/l), and a low normal FT 4 concentration (2.7 mU/l, IGF-I). MRI revealed anterior pituitary hypoplasia, with a normal posterior pituitary and infundibulum (Fig. 2F).

His father (II.3, Fig. 2A) had previously presented at the age of 6 years with short stature (height 96 cm (−5.23 SDS), weight 14 kg (−2.66 SDS), mid-parental height 173 cm (−0.3 SDS)). He was born at term with a birth weight of 3.9 kg (0.7 SDS). On examination at presentation, facial features were suggestive of GHD (Fig. 2D). The bone age was delayed by 2 years. GH provocation (insulin-induced hypoglycemia) revealed GHD (GH peak 3 mU/l) with a basal cortisol of 151.8 nmol/l. Treatment with pituitary-derived hGH was commenced at the age of 9 years, and his height velocity improved from 3.5 to 8.6 cm/year. He underwent spontaneous puberty with 12 and 15 ml testicular volumes. He achieved a final height of 164.3 cm (−1.6 SDS) with a weight of 49.8 kg (−1.8 SDS) at the age of 19 years. At the end of GH treatment, re-testing confirmed profound GHD with normal thyroid function and normal cortisol and gonadotropin responses to provocation.

He was lost to follow-up for a number of years, and re-presented with his son III.2 at the age of 38 years, having been untreated for 20 years. He complained of lack of stamina with extreme fatigue, poor libido, and recognized difficulties in coping with work. During the subsequent evaluation, he also reported secondary infertility. Examination revealed centripetal obesity and the typical facial features of an adult with GHD (Fig. 2E). His testicular volumes were 15 ml bilaterally. A combined pituitary function test was performed (Table 1), revealing severe GHD (peak GH < 0.6 mU/l), a borderline cortisol response (peak cortisol to insulin-induced hypoglycemia 502.3 nmol/l), a suboptimal prolactin response (peak prolactin 262 mU/l) to TRH, and low basal gonadotropins with a poor LH response to LHRH (basal LH 1.5 U/l, FSH 1.0 U/l; peak LH 11.7 U/l (NR. 12–29.7 U/l), peak FSH 4.0 U/l (NR. 2.9–7.8 U/l)) with a low testosterone (9.7 nmol/l). He was also noted to have hypercholesterolemia (fasting cholesterol 7.3 mmol/l (NR 1–5 mmol/l); triglyceride 4.4 mmol/l (NR 0.5–2.0 mmol/l)). MR scanning revealed a pituitary fossa of relatively small volume, with only a small rim of pituitary tissue in its floor (Fig. 2G), a normal infundibulum and a eutopic posterior pituitary.

The father’s quality of life was substantially impaired as determined by a Quality of Life-Assessment of Growth Hormone Deficiency in Adults (QoL-AGHDA) score of 18/25 reflecting a high number of adverse symptoms (19), and he was therefore commenced on rhGH treatment. This initially led to carpal tunnel syndrome, but a reduction in hGH dose was associated with the resolution of these symptoms. His QoL-AGHDA score decreased to 10 on GH treatment, consistent with a marked improvement in his symptoms. The testosterone concentration (0900 h) decreased further to 6.6 nmol/l, with a nadir of 4.0 nmol/l at the age of 40.8 years. Semen analysis showed a normal sperm count (100 million/ml) but with an abnormally high proportion (92%) of abnormal forms. He was commenced on testosterone (testogel 25 mg od) replacement. This led to improvement in his muscle bulk, stamina, libido, hair growth, mood, and work performance. It is, therefore, conceivable that the high QoL-AGHDA score prior to treatment may reflect not only the GHD, but also gonadotropin deficiency. However, his sperm count decreased further to 1 million/ml on this replacement therapy. Gonadotropin treatment is presently in progress in an attempt to achieve fertility.

Genomic analysis of GH-1

Analysis of the GH-1 gene identified a heterozygous change at IVS-3+1 that changes the G of the invariant GT dinucleotide splice donor site to an A nucleotide (Fig. 3), first described by Cogan et al. (20) (Online Mendelian Inheritance in Man #139250.0009). No other sequence variants were identified in the proband or his father.

Discussion

Human IGHD type II also shows a marked degree of variability in onset, severity and progression, even within the same family (21), with only a third of the affected individuals manifesting severe short stature at diagnosis. Overall, the incidence of these mutations is low; we have now screened 88 IGHD patients for mutations in GH-1, and identified five patients with IVS 3 mutations, giving a frequency of 5.7% (our unpublished data). Mullis et al. have recently shown a co-existing variable partial ACTH and TSH insufficiency in some of these individuals (13). Additionally, the
Figure 2 (A) Pedigree of family with IVS-3 + 1G > A mutation in GH-1. Members II.3 and III.2 are affected with isolated growth hormone deficiency (IGHD). (B) Growth chart of III.2 showing the classical growth pattern of GHD with a good response to rhGH. (C) Phenotype of III.2 on presentation. (D) Phenotype of II.3 on presentation. (E) Phenotype of II.3 at re-presentation. (F) MRI of pituitary in III.2 showing anterior pituitary hypoplasia and a normally placed posterior pituitary with a normal infundibulum. (G) MRI of pituitary in II.3 showing anterior pituitary hypoplasia and a normally placed posterior pituitary with a normal infundibulum.
pituitary heights on MRI were lower in these patients, suggesting a degree of hypoplasia. None of the individuals in this study was reported as showing evidence of gonadotropin deficiency, although detailed assessment of the hypothalamo–pituitary–gonadotrope axis was not performed, and the diagnosis of ACTH deficiency was made on the basis of a low fasting cortisol as measured at 0800 h, as opposed to a provocative test of cortisol secretion.

We have now shown that IGHD type II mutations can be associated with an evolving gonadotropin deficiency. We report a pedigree whereby a father and son presented with severe short stature due to profound GHD characterized by a poor GH response to provocation with low concentrations of IGF-I and IGFBP-3. The father, II.3, was initially treated with pituitary-derived GH, but was later untreated over a 20-year period. On reassessment, he showed evidence of profound clinical and biochemical GHD in addition to profound pituitary hypoplasia and secondary to evolving GHD. Another factor is a reduced gonadotropin deficiency, although detailed assessment of the hypothalamo–pituitary–gonadotrope axis was not performed, and the diagnosis of ACTH deficiency was made on the basis of a low fasting cortisol as measured at 0800 h, as opposed to a provocative test of cortisol secretion.

We have now shown that IGHD type II mutations can be associated with an evolving gonadotropin deficiency. We report a pedigree whereby a father and son presented with severe short stature due to profound GHD characterized by a poor GH response to provocation with low concentrations of IGF-I and IGFBP-3. The father, II.3, was initially treated with pituitary-derived and recombinant hGH, but was later untreated over a 20-year period. On reassessment, he showed evidence of profound clinical and biochemical GHD in addition to partial prolactin, ACTH (after insulin-induced hypoglycemia) and gonadotropin deficiencies with low concentrations of IGF-I and IGFBP-3. The father, II.3, was initially treated with pituitary-derived and recombinant hGH, but was later untreated over a 20-year period. On reassessment, he showed evidence of profound clinical and biochemical GHD in addition to partial prolactin, ACTH (after insulin-induced hypoglycemia) and gonadotropin deficiencies. Small amounts of GH have been reportedly expressed ectopically in other pituitary cell types in rodents, notably gonadotropes (23), but for an effective dominant effect, this would have to be at a significant protein level. We believe this is unlikely, especially since small amounts of 17.5 kDa transcript and product are detectable endogenously in normal pituitaries (24). It seems more likely that a phenomenon observed in transgenic mice (17), namely an invasion by activated macrophages, may lead to significant bystander endocrine cell killing, which in turn compromises cellular repletion of the other lineages, and ultimately to additional endocrine deficits.

In theory, removal of the GHRH drive to generate greater quantities of both wild-type and mutant GH could potentially ameliorate the phenotype and prevent
the evolution of other endocrinopathies. Indeed, it is interesting to note that patient II.3 had been untreated for 20 years when he re-presented with hypopituitarism. However, Mullis et al. concluded that treatment of the patients with rhGH made no difference to the evolution of the hypopituitarism (13).

This family study illustrates the additional insights gained by precise genetic diagnosis in subjects with apparently isolated GHD. From a clinical perspective, it is evident that evaluation of pituitary function in those individuals are at risk of evolving pituitary endocrinopathy. We suggest that all individuals with AD IGHD should be screened at annual intervals (0800 h cortisol, testosterone). Additionally, although the evolution of the phenotype has only been described in association with the IVS-3+1G→A mutation in II.2 (top panel) and in II.3 (middle panel) compared with wild-type sequence (bottom panel).

Acknowledgements

Mehul T Dattani and James PG Turton were supported by an MRC Career Establishment Grant.

References

9 Moseley CT, Moseley CT, Mulley PE, Prince MA & Phillips JA, III. Moseley CT, Magoulas CB, Robinson IC & Patton JG. Disruption of exon definition produces a dominant-negative growth hormone isoform that causes somatotroph death and IGHD II. Human Genetics 2003 113 140–148.


**Received** 19 June 2006
**Accepted** 12 September 2006