

## LETTERS TO THE EDITOR

**Pseudohypoparathyroidism type 1a due to a novel mutation in the *GNAS* gene**

Dear Editor,

Pseudohypoparathyroidism type 1a (PHP1a) (OMIM #103580) is characterized by hypocalcaemia and hyperphosphataemia due to parathyroid hormone (PTH) resistance, associated with features of Albright's Hereditary Osteodystrophy (AHO) which include short stature, obesity, subcutaneous calcifications and brachydactyly.<sup>1</sup> PHP1a is caused by heterozygous germline mutations of the alpha subunit of the stimulatory form of the GTP-binding protein (Gs-alpha), which is a downstream signalling protein of the PTH receptor and of other G protein-coupled hormone receptors.<sup>1</sup> Gs-alpha is encoded by the *GNAS* gene (chromosome 20q13.3), which is a complex imprinted locus that also produces additional coding and noncoding transcripts through the use of alternative promoters and alternative splicing, in a tissue-specific manner.<sup>2</sup> PHP1a results from maternally inherited loss-of-function mutations of Gs-alpha, but paternally inherited mutations usually result in pseudopseudohypoparathyroidism (PPHP), which is characterized by the presence of AHO without hormone resistance. Thus, PHP1a and PPHP, which are inherited as autosomal dominant disorders with parental imprinting, are frequently found in the same kindred.

We identified a kindred with PHP1a/PPHP and investigated the patients for the underlying molecular abnormality. The index case was an 11-year-old Portuguese boy that presented with seizures due to hypocalcaemia. Serum concentration of calcium was 1.65 mmol/l (normal: 2.0–2.6 mmol/l), phosphate 3.6 mmol/l (normal: 0.9–1.5 mmol/l) and PTH 607 ng/l (normal: 9–55). Height and weight were on the 50th percentile and 90th percentile, respectively. He had subcutaneous and intracranial calcifications (Fig. 1a); brachydactyly, which was due to shortened metacarpals and metatarsals (Fig. 1b); and learning disability. He also had abnormal thyroid function tests with serum concentrations of TSH 4.8 mIU/l (normal: 0.3–3.0), free T4 6.4 pmol/l (normal: 7.7–23.2) and free T3 6.6 pmol/l (normal: 3.4–8.4). His mother had short stature (3rd percentile) and brachydactyly (Fig. 1c), but no biochemical abnormalities. Family history also included an older sibling with a similar phenotype, who had died due to an unknown cause, at age 7 years, and two younger unaffected siblings.

Venous blood samples were obtained after informed consent, from the index case, the mother, two siblings and the maternal grandparents. Leucocyte DNA was extracted and used with appropriate PCR primers to amplify exons 1–13 of the *GNAS* gene, utilizing conditions previously described.<sup>3</sup> Bidirectional sequencing of the PCR products was carried out by the use of the same primers, and an automated capillary sequencer (GenomeLab GeXP System, Beckman Coulter, Fullerton, CA, USA).

A novel heterozygous 2-base pair (bp) deletion in exon 2 (c.188\_189delTG) was found in the proband and his mother (Fig. 1d). The deletion of this dinucleotide sequence is predicted to cause a frameshift, with the incorporation of two missense amino acids, followed by a premature stop codon (TAA) in the new frame at codon 65. The mutation resulted in the loss of an *NspI* restriction endonuclease site and this was used to confirm its presence and to assess the other family members (Fig. 1e). The mutation was absent in the unaffected family members, including the maternal grandparents, thereby indicating that the mother (individual II-2 in Fig. 1e) either has a *de novo* mutation involving the paternal allele or that her father has undetected mosaicism. In addition, an analysis of the DNA from 55 unrelated normal individuals (110 alleles) confirmed the absence of this DNA sequence abnormality (data not shown). The different clinical presentation in the son (PHP1a) and mother (PPHP), who harbour the same mutation, can be explained by the characteristic mode of inheritance, which is autosomal dominant with parental imprinting of hormone resistance.

The identification of the causative mutation in the index case may be useful for screening other family members in order to avoid late or misdiagnosis, as probably occurred with individual III-1 (Fig. 1e). Age of onset of the hormone resistance is quite variable among mutation-positive individuals and can be delayed for several years. This latency of PTH resistance in patients with PHP1a has been attributed to a gradual development of paternal Gs-alpha silencing in target tissues.<sup>4</sup> Therefore, genetic screening of family members can be useful for presymptomatic diagnosis.

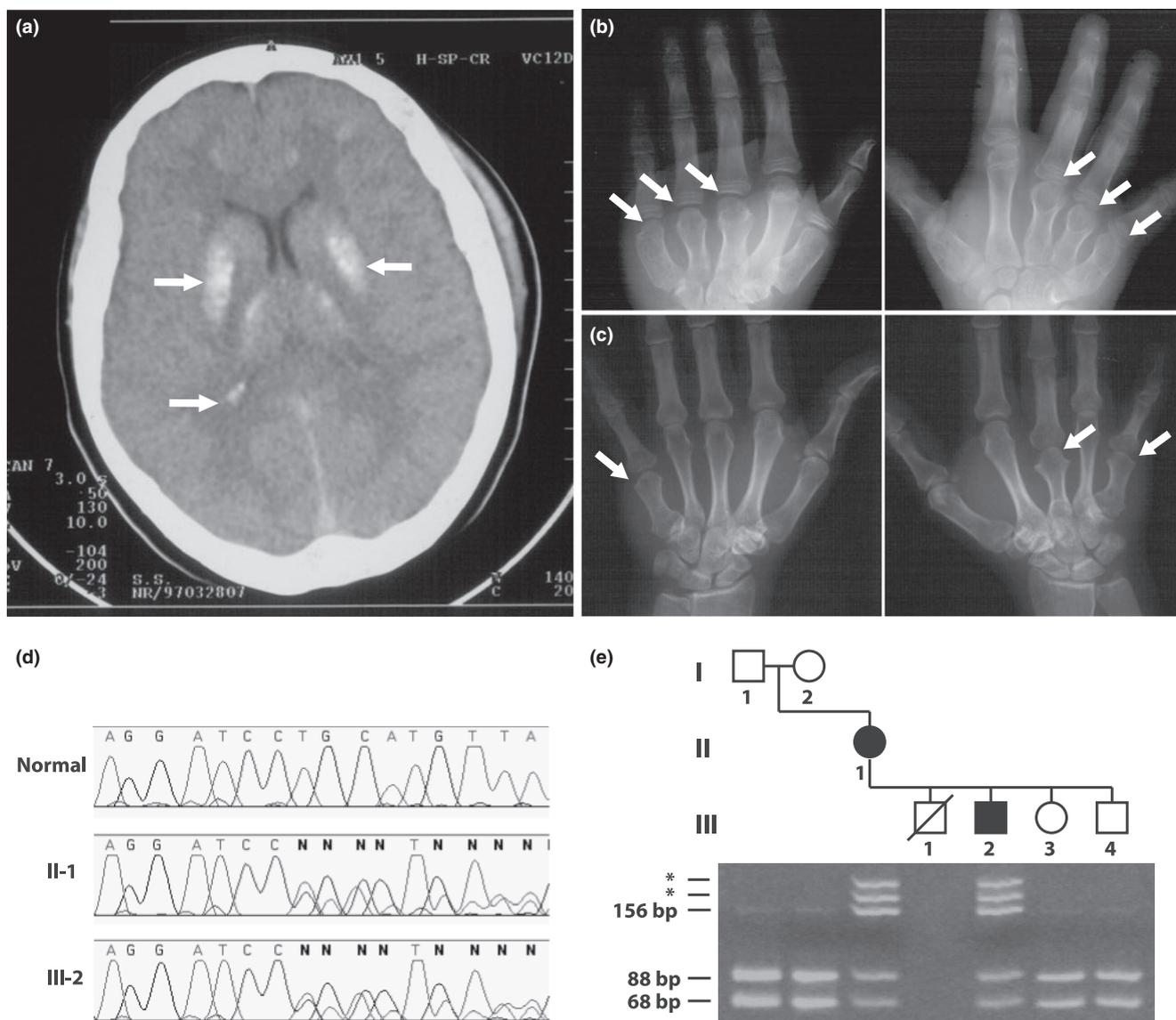
There are over 340 reported kindreds with PHP1a/PPHP due to a *GNAS* mutation,<sup>5</sup> and these are scattered across the 13 exons that encode Gs-alpha, with no known genotype–phenotype correlation. However, exons 2 and 3 are the least affected in these disorders. To date, only three other mutations of exon 2, one missense and two insertions, have been reported.<sup>5</sup> Therefore, the 2-bp deletion in exon 2 identified by the present study is unusual and further expands the spectrum of known *GNAS* mutations associated with these complex disorders.

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**Fig. 1** (a) Head computed tomography (CT) scan of the affected child showing calcification of basal ganglia and of other dispersed regions of the brain (arrows). (b) Radiograph showing shortening bilaterally of the 3rd, 4th and 5th metacarpals (arrows) in the affected child, typical of Albright's osteodystrophy. (c) Shortening of the left 5th, and right 3rd and 5th metacarpals in the mother who has pseudopseudohypoparathyroidism (PPHP). (d) DNA sequence analysis of both mother (II-1) and child (III-2) revealed a frameshift that resulted from a heterozygous 2-base pair (bp) deletion at codon 63 (c.188\_189delTG). The mutation nomenclature was based on the *GNAS* cDNA reference sequence (GenBank Accession number NM\_000516.4). (e) This 2-bp deletion resulted in the loss of an *NspI* restriction enzyme site in the mutant sequence, and this facilitated the screening of the mutation in other family members. Electrophoresis of *NspI* digested PCR fragments on an 8% polyacrylamide gel showed that one product of 156 bp was obtained from the mutant sequence (additional heteroduplex products are indicated by asterisks), but two products of 88 and 68 bp were obtained from the wild-type normal sequence. The affected individuals are heterozygous, and the unaffected are homozygous for the wild-type allele. Individuals are represented as males (squares), females (circles), unaffected (open symbol), affected (filled symbol) and deceased (oblique line through symbol). Individual III-1 was reported to share a similar phenotype as III-2 by his relatives.

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### Increasing severity of traumatic brain injury in early childhood is associated with a progressive reduction in long-term serum thyroid-stimulating hormone concentrations

Structural traumatic brain injury (TBI) can result in late-occurring health sequelae, consisting mainly of neuroendocrine dysfunctions.<sup>1,2</sup> Studies have suggested that hypopituitarism is relatively common following TBI in childhood, but recent evidence suggests that the incidence appears to be frequently overestimated.<sup>3</sup> We recently showed that permanent hypopituitarism is rare after both inflicted and accidental structural TBI in early childhood.<sup>3</sup>

However, subtle disturbances in pituitary function have been reported after TBI, including abnormalities in thyroid function.<sup>2</sup> Niederland and colleagues found that concentrations of peripheral thyroid hormones were lower in children with TBI history than in control subjects, even though their levels were in age-related normal ranges.<sup>4</sup> A recent small pilot study reported a subtle reduction in overnight thyroid-stimulating hormone (TSH) surge in children following moderate-to-severe inflicted TBI.<sup>1</sup> Thus, it is unclear whether injury severity can be responsible for more subtle long-term effects on thyroid function in the absence of hypopituitarism. Therefore, we assessed whether severity of TBI was associated with changes in circulating thyroid hormone concentrations in childhood in a cross-sectional study.

Ethics approval was provided by the Northern X Regional Ethics Committee. This study covers a previously reported cohort of children who suffered TBI in Auckland (New Zealand).<sup>3</sup> Cases were eligible if structural TBI had occurred within the first 5 years of life, and more than 12 months previously. Structural TBI was defined as the presence of skull fracture, intracranial haemorrhage (extradural, subdural, subarachnoid or intraventricular) or cerebral injury (contusion, infarct, oedema or diffuse axonal injury) reported on computerized tomography or magnetic resonance imaging scan. Structural TBI was graded according to the Abbreviated Injury Scale for the head region (AIS-HR).<sup>5</sup> AIS-HR is an anatomical scoring system, where injuries are ranked on a ‘threat to life’ scale of

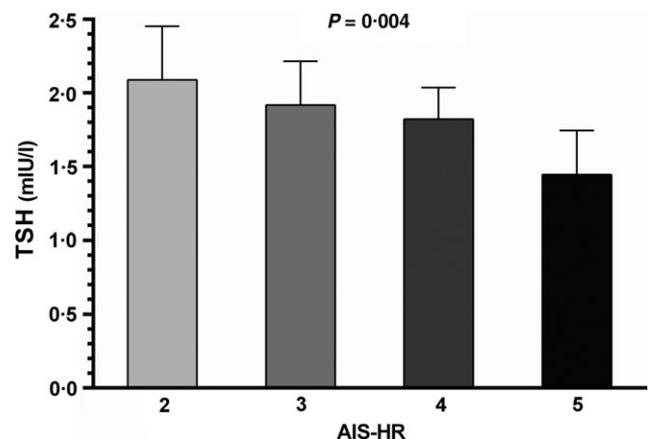
1–6 (1 – mild, 2 – moderate, 3 – serious, 4 – severe, 5 – critical and 6 – not survivable).<sup>5</sup> All participants had structural TBI with AIS-HR  $\geq 2$ .

Participants underwent a single clinical assessment at the Maurice and Agnes Paykel clinical research unit at the Liggins Institute (University of Auckland). All assessments were carried out between 8:00 and 10:00 am, after a 15-min period of rest.<sup>6</sup> Assays were performed as previously described.<sup>6</sup>

One hundred and ninety-eight (112 males) survivors of structural TBI sustained in early childhood [age at injury 1.7 years (SD = 1.5)] were assessed 6.5 years (SD = 3.2) after injury. No participants had significant abnormalities in thyroid function. Three participants had slightly elevated TSH concentrations that were 0.8–1.5 mIU/l above the normal range (0.50–4.70 mIU/l), but all displayed normal fT3 (1.5–9.2 pmol/l), fT4 (10–26 pmol/l) and prolactin (40–600 mIU/l) concentrations.

Greater AIS-HR scores (i.e. increasing TBI severity) were correlated with decreasing TSH concentrations ( $\rho = -0.20$ ;  $P = 0.004$ ), but not with free triiodothyronine (fT3;  $P = 0.10$ ) or free thyroxine (fT4;  $P = 0.49$ ) concentrations. Multivariate analyses (adjusting for important confounders) showed that increasing TBI severity was associated with a progressive reduction in serum TSH concentrations ( $P = 0.004$ ; Fig. 1). Thus, children with an AIS-HR score of 2 had a mean TSH concentration of 2.09 (95% CI 1.78–2.45) mIU/l compared to 1.44 (95% CI 1.20–1.74) mIU/l for those with an AIS-HR of 5 ( $P = 0.002$ ), that is TSH concentrations were 31% lower in the most severe TBI cases.

Overall, increasing TBI severity was not associated with changes in fT3 ( $P = 0.17$ ) or fT4 ( $P = 0.77$ ) concentrations. However, the most severe TBI cases (AIS-HR = 5) had lower fT3 concentrations than the rest of the cohort: 6.29 (95% CI 5.95–6.64) vs 6.64 (95% CI 6.47–6.82) pmol/l ( $P = 0.046$ ).



**Fig. 1** The association between severity of structural traumatic brain injury (TBI) and thyroid-stimulating hormone (TSH) levels in 198 children. Data are means and 95% confidence intervals adjusted for confounding factors in the multivariate models: sex, pubertal status, ethnicity, age at assessment, time lag between injury and assessment, BMI SDS and TBI class (accidental vs inflicted). Higher Abbreviated Injury Scale for the head region (AIS-HR) scores represent more severe injury.  $P$ -value shown is for a continuous association.