

# Novel *TSHB* variant (c.217A>C) causing severe central hypothyroidism and pituitary hyperplasia

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## Summary

Biallelic pathological variants in the thyroid stimulating hormone (TSH) subunit  $\beta$  gene (*TSHB*) result in isolated TSH deficiency and secondary hypothyroidism, a rare form of central congenital hypothyroidism (CCH), with an estimated incidence of 1 in 65 000 births. It is characterised by low levels of free thyroxine and inappropriately low serum TSH and may therefore be missed on routine neonatal screening for hypothyroidism, which relies on elevated TSH. We describe a patient with CCH who developed recurrence of pituitary hyperplasia and symptomatic hypothyroidism due to poor compliance with thyroxine replacement. She was diagnosed with CCH as a neonate and had previously required trans-sphenoidal hypophysectomy surgery for pituitary hyperplasia associated with threatened chiasmal compression at 17 years of age due to variable adherence to thyroxine replacement. Genetic testing of *TSHB* identified compound heterozygosity with novel variant c.217A>C, p.(Thr73Pro), and a previously reported variant c.373delT, p.(Cys125Valfs\*10). Continued variable adherence to treatment as an adult resulted in recurrence of significant pituitary hyperplasia, which subsequently resolved with improved compliance without the need for additional medications or repeat surgery. This case describes a novel *TSHB* variant associated with CCH and demonstrates the importance of consistent compliance with thyroxine replacement to treat hypothyroidism and prevent pituitary hyperplasia in central hypothyroidism.

## Learning points:

- Pathogenic variants in the TSH subunit  $\beta$  gene (*TSHB*) are rare causes of central congenital hypothyroidism (CCH).
- c.217A>C, p.(Thr73Pro), is a novel *TSHB* variant, presented in association with CCH in this case report.
- Thyroxine replacement is critical to prevent clinical hypothyroidism and pituitary hyperplasia.
- Pituitary hyperplasia can recur post-surgery if adherence to thyroxine replacement is not maintained.
- Pituitary hyperplasia can dramatically reverse if compliance with thyroxine replacement is improved to maintain free thyroxine (FT4) levels in the middle-to-upper normal range, without the need for additional medications or surgeries.

## Background

Central congenital hypothyroidism (CCH) is a rare form of hypothyroidism, with an estimated prevalence of approximately 1 in 16 000–30 000 live births. CCH is characterised by low levels of free thyroxine (FT4) and

inappropriately low serum thyroid-stimulating hormone (TSH).

CCH commonly occurs in association with other pituitary hormone deficits due to mutations in pituitary



transcription factors. However, isolated TSH deficiency causing secondary hypothyroidism is particularly rare (OMIM: 275100), estimated between 1 in 65 000 births (1). Known genetic causes include defects in TSH signalling genes of the TSH subunit  $\beta$  (*TSHB*; OMIM: 188540), thyrotropin releasing hormone receptor, and immunoglobulin superfamily member 1 (*IGSF1*).

TSH is a member of the glycoprotein hormone family, which includes luteinising hormone, follicle-stimulating hormone, and human chorionic gonadotropin. All are heterodimers of a common  $\alpha$  subunit and subtype-specific  $\beta$  subunit, which determines the biologic specificity. Therefore, biallelic pathological mutations in *TSHB* result in severe isolated TSH deficiency. In this report, we present a patient with CCH due to two compound heterozygote pathogenic variants in *TSHB*, with subsequent thyrotrope hypertrophy which occurred during several periods of non-adherence to thyroxine replacement therapy.

## Case presentation

A 31-year-old Caucasian woman was referred to our Endocrinology department in October 2013 for ongoing management of TSH-deficient CCH. She was diagnosed with CCH as a neonate during investigation of neonatal jaundice. Her thyroid function tests showed a total T4 of 39 nmol/L (reference range: 126–214 nmol/L) and TSH which was unmeasurable by RIA. Anterior and posterior pituitary function tests were otherwise normal, and a brain CT showed no anomaly of the hypothalamus or pituitary. On full replacement thyroxine throughout infancy and childhood, she grew and developed normally and was supported very well by her parents who are nonconsanguineous. Aged 17 years of age, she had undergone trans-sphenoidal hypophysectomy to treat pituitary hyperplasia associated with headaches and threatened chiasmal compression. In retrospect, she admitted she was variably compliant with thyroxine replacement in her teenage years. She continued thyroxine replacement and was otherwise eupituitary following surgery.

In October 2013, she presented with an episode of muscle pain, fatigue, and headaches, associated with hypothyroidism, again due to variable adherence to her thyroxine replacement therapy. At the time, she was taking 250  $\mu$ g daily but admitted missing 2–3 days of treatment per week. She took no other medications. She was otherwise well and reported regular menses. There was no family history of endocrine disease. On examination, her weight was stable compared to previous visits at 78.8 kg. She was in

sinus rhythm with blood pressure 95/60. The thyroid was impalpable. Visual fields were normal. The remainder of the examination was normal.

## Investigation

The patient was markedly hypothyroid, with low TSH (0.025 mIU/L; reference range: 0.40–3.50 mIU/L), low free T4 (FT4, 6.5 pmol/L; reference range: 9.0–19.0 pmol/L), and low free T3 (FT3, <1.5 pmol/L; reference range: 2.6–6.0 pmol/L), consistent with non-adherence to thyroxine replacement. Creatine phosphokinase (CPK) was elevated at 690 U/L (reference range: 29–168 U/L), consistent with myositis associated with severe hypothyroidism. Prolactin was slightly elevated at 616 mIU/L (reference range: 110–560 mIU/L), which can occur in severe hypothyroidism from stimulation by thyrotropin-releasing hormone (TRH). C-reactive protein was normal (0.6 mg/L), and full blood count was normal except for mild eosinophilia ( $0.5 \times 10^9/L$ ). B12 and iron studies were normal, and coeliac serology was negative.

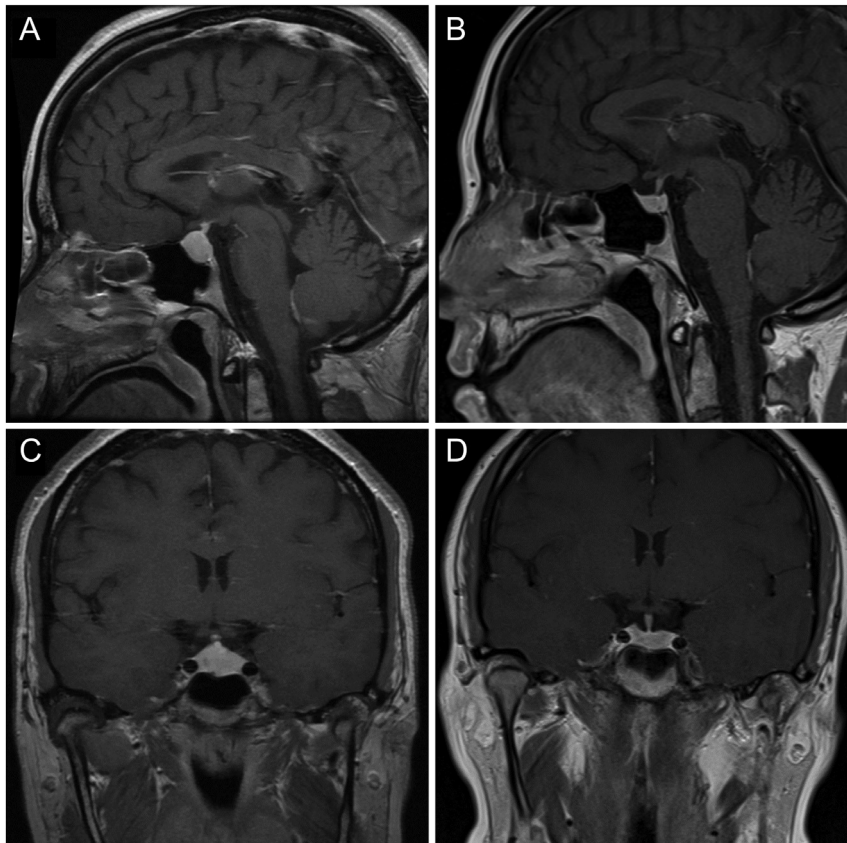
MRI pituitary demonstrated marked hyperplasia extending above the sella, but clear of the optic chiasm, with the pituitary height measuring 13 mm (Fig. 1A and C).

To determine the cause of her isolated TSH deficiency and CCH, genetic testing of *TSHB* was performed. Sanger sequencing revealed compound heterozygosity for a previously described variant c.373delT, p.(Cys125Valfs\*10), and a novel variant c.217A>C, p.(Thr73Pro), not previously described in the literature, not present in gnomAD v3.1.2., and not listed in ClinVar (Fig. 2). *TSHB* protein sequence homology of the region including Thr73 was analysed using HomoloGene. The region was highly conserved across species, with Thr73 and the four amino acids on either side of it conserved down to *Rattus norvegicus* and the threonine itself retained down to *Danio rerio* (Fig. 3).

MutationTaster2021 was used to assess conservation of the local genetic sequence. Conservation was suggested for both mutations, with positive PhyloP scores of 1.221 and 3.647 for c.217A>C and c.373delT, respectively, with both scoring 1 on PhastCons (2). The impact on protein function due to amino acid substitution in novel variant c.217A>C was assessed using *in silico* tools, with PROVEAN (score: –5.50) and SIFT (score: 0.001) predictions suggesting the change would be deleterious or damaging, respectively.

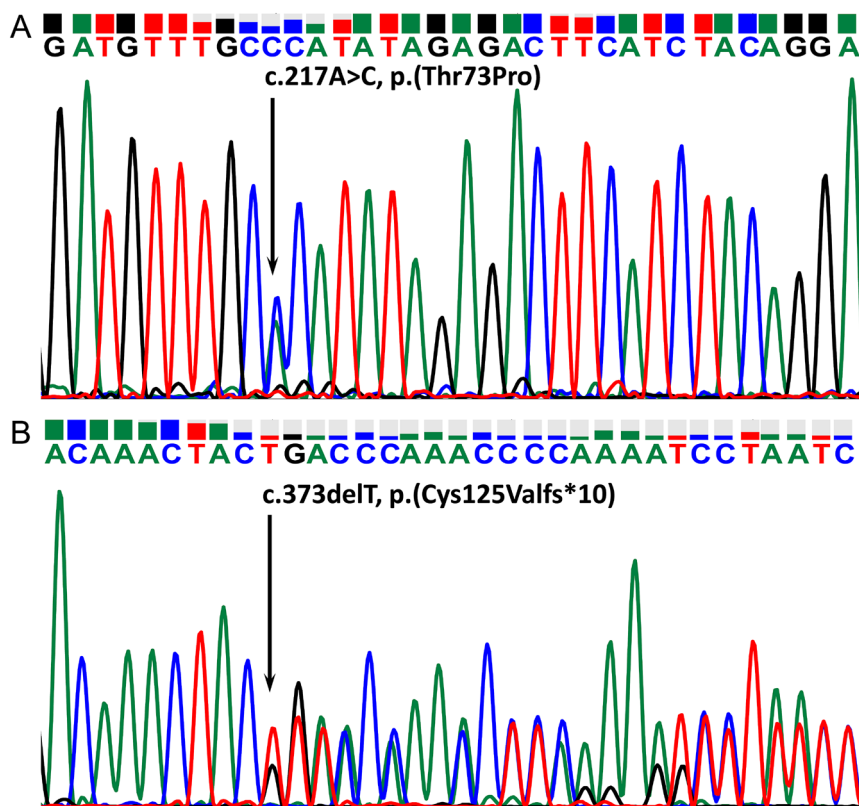
## Treatment

She was recommended to strictly adhere with her thyroxine replacement therapy (250  $\mu$ g daily). Patient



**Figure 1**

T1-weighted MRI images (post contrast) of pituitary fossa in transverse and sagittal section from October 2013 (A and C) and June 2017 (B and D). Reduction in pituitary size is evident, following resumption of consistent thyroxine replacement.



**Figure 2**

Electropherograms of *TSHB* exon 2 variants in compound heterozygous proband. (A) Single nucleotide base missense variant, (B) single nucleotide deletion nonsense variant.

| Species                   | Amino acid sequence (TSHB) <sup>a</sup>    |
|---------------------------|--|
| <i>Homo sapiens</i>       | 68 SQDVCT <b>TY</b> RDFIYR 80 <sup>β</sup> |
| <i>Pan troglodytes</i>    | 88 SQDVCT <b>TY</b> REFIYR 100             |
| <i>Macaca mulatta</i>     | 88 SQDVCT <b>TY</b> RDFIYR 100             |
| <i>Canis lupus</i>        | 68 SQDVCT <b>TY</b> RDFMYK 80              |
| <i>Bos taurus</i>         | 68 SQDVCT <b>TY</b> RDFMYK 80              |
| <i>Mus musculus</i>       | 68 SQDVCT <b>TY</b> RDFIYR 80              |
| <i>Rattus norvegicus</i>  | 68 SQDVCT <b>TY</b> RDFTYR 80              |
| <i>Gallus gallus</i>      | 68 SQNVCT <b>TY</b> KEMFYQ 80              |
| <i>Danio rerio</i>        | 67 VQRGCT <b>TY</b> QVEVEYR 79             |
| <i>Xenopus tropicalis</i> | 68 SQKVCSYKQYIHR 80                        |

**Figure 3**

Comparison of TSHB amino acid sequence surrounding threonine at codon 73 (bolded) across Euteleostomi. <sup>a</sup>Created using HomoloGene (NCBI), listed in reducing DNA similarity from humans (*Homo sapiens*). <sup>β</sup>Numbers flanking the amino acid sequences represent codon position.

education was provided with emphasis on the importance of compliance with thyroxine replacement, for adequacy of thyroid hormone effect and to prevent further pituitary enlargement.

### Outcome and follow-up

The patient presented for routine follow-up 7 months later in May 2014 and again in June 2017. Following improved compliance with thyroxine, her muscle aches had resolved

in 2014, with normalisation of CPK (35 U/L), and she was otherwise in good health. She retained a regular menstrual cycle, though she continued to report occasional central headaches.

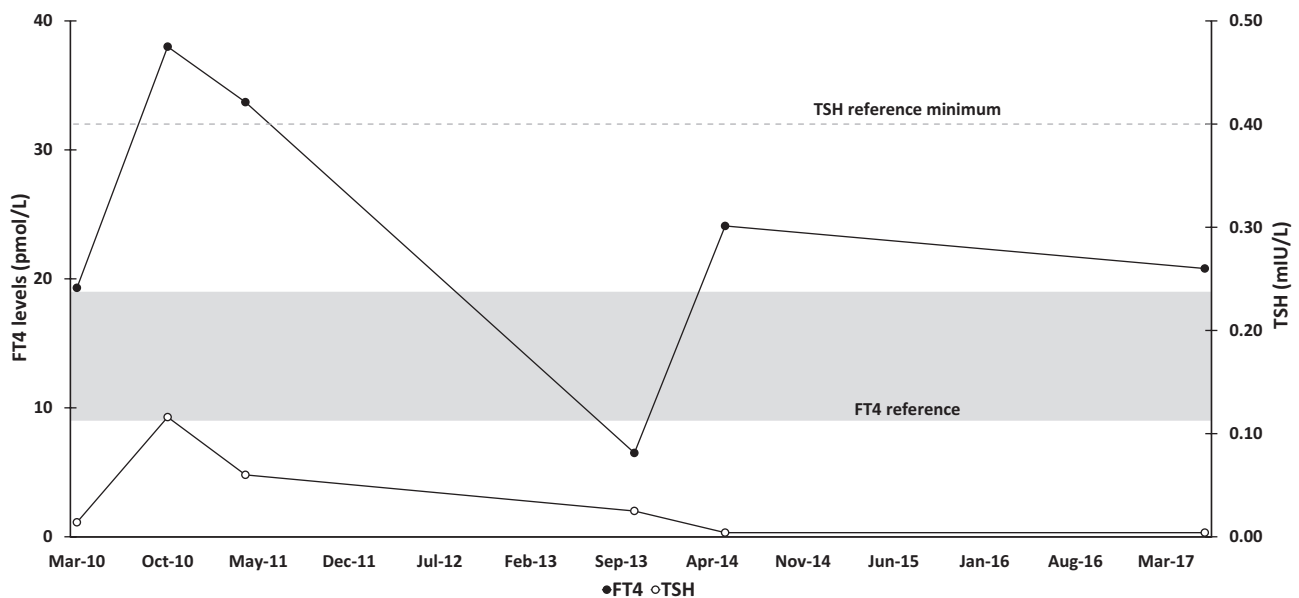
She was now adherent with her thyroxine therapy, and serum FT4 increased from below to just above the reference range; serum TSH has since remained below the reference range (Fig. 4). Comparison of her historic TSH and FT4 measures indicated that TSH did not rise as FT4 fell from October 2010 to October 2013, preceding her symptomatic hypothyroid presentation we describe in this case report, indicating that TSH elevation was not a reliable marker of under-replacement.

MRI imaging of the pituitary was repeated in June 2017, which demonstrated normal pituitary size (Fig. 1B and D). Blood results also demonstrated normalisation of prolactin to 140 mIU/L (reference range: 110–560 mIU/L).

### Discussion

We present a patient with a novel compound heterozygous pathogenic *TSHB* variant (c.217A>C) causing central hypothyroidism and subsequent thyrotrope hyperplasia. Notably, this case describes relapse of pituitary hyperplasia following surgical debulking due to a period of non-adherence with thyroxine replacement.

*TSHB* is a 4.5 kb gene located on chromosome 1 (1p13.2) and is comprised of 3 exons. Exons 2 and 3 encode



**Figure 4**

TSH and free T4 (FT4) hormone serum concentrations over time in our patient. TSH measurements are plotted according to the right y-axis, with FT4 levels plotted according to the left y-axis. Laboratory reference ranges for FT4 (9.0–19.0 pmol/L) and TSH (0.40–3.50 mIU/L) are shown.



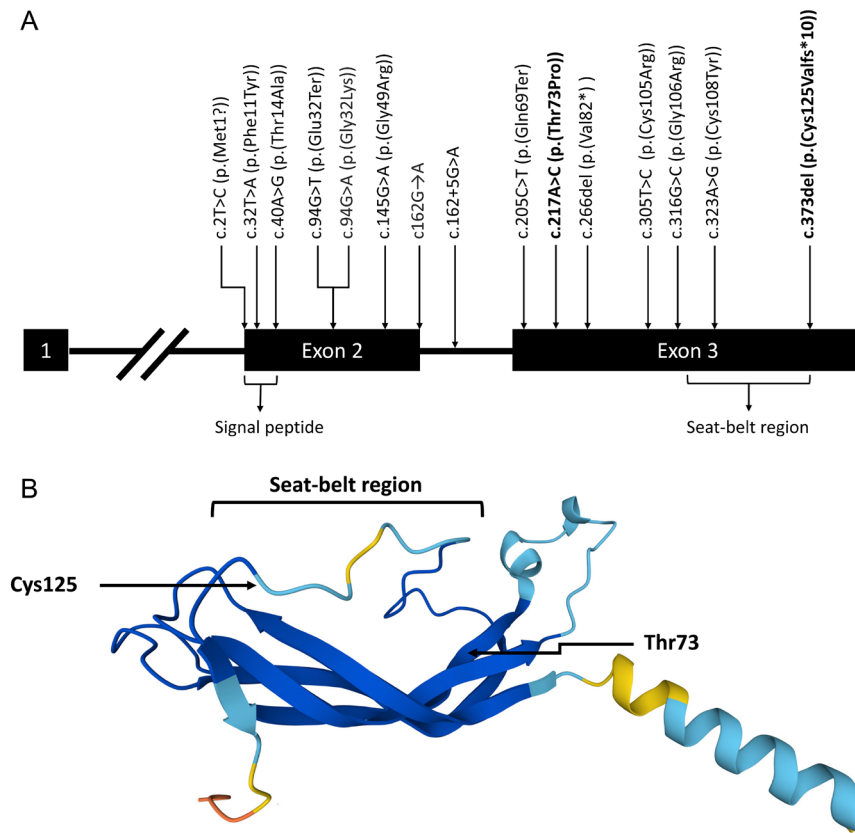
a 138 amino acid sequence, including a 20 N-terminal amino acid signal peptide and 6 amino acid C-terminal extension, which are cleaved to yield a mature 112 amino acid TSHB protein. Development of CCH requires biallelic loss-of-function in *TSHB* and follows an autosomal recessive pattern of inheritance (3).

To date, 14 *TSHB* variants implicated in CCH have been reported in the literature and are illustrated in Fig. 5. Variants typically disrupt TSHB function through significant truncation following frameshift, nonsense, or splice-site mutations or by disrupting TSH dimerization due to missense mutations in the ‘seat-belt’ region of TSHB, which is involved in forming multiple disulphide bonds with TSHA. *TSHB* allele deletions, and variants involving the signal peptide sequence, have also been implicated in CCH.

The c.373delT (p.(Cys125Valfs\*10)) variant (legacy numbering: c.313delT, p.C105Vfs114X) involves a thymine deletion at nucleotide 373 which causes a cysteine to valine change at the critical Cys125 located in the seat-belt region, which normally forms a disulphide bridge with Cys39, likely impairing dimerization with TSHA (4). Resultant frameshift also results in a premature stop codon at position 134. It is the most commonly reported pathogenic variant causing CCH, and it has been identified

throughout continental Europe, the Americas, and the United Kingdom. Notably, this is the first report of the variant in Australasia, albeit our patient comes from European (non-Finnish) ancestry. While many *TSHB* variants are most common in consanguineous families, the vast majority of c.373delT variant cases have been reported in unrelated and nonconsanguineous families (5), and haplotype analysis has suggested a founder effect, with monophyletic origin of the mutation (6). Notably, in gnomAD (version 3.1.2), this variant is found in European (non-Finnish) at an allele frequency of 0.0003088 and is listed in ClinVar as pathogenic/likely pathogenic and high-confidence putative loss-of-function.

Compound heterozygote cases involving c.373delT and a second pathogenic variant have previously been reported, resulting in CCH of equal severity to c.373delT homozygote cases (7). Here, we present a patient with novel c.217A>C (p.(Thr73Pro)) variant in combination with c.373delT, resulting in neonatal-onset CCH, suggesting pathogenicity of the c.217A>C variant. The missense change to proline would be predicted to confer significant conformational change, and threonine at codon 73 lies within a highly conserved region of *TSHB*. Our assessment for pathogenicity of this novel variant is ‘likely pathogenic,’ according to American College



**Figure 5** Summary of *TSHB* variants in patients with central congenital hypothyroidism (CCH). (A) Schematic illustrating approximate locations of pathogenic *TSHB* variants as reported in the literature and in this case report (bolded); and (B) location of the amino acids involved in the *TSHB* variants reported in this case report, on a 3D model of the TSHB protein accessed from UniProt (SMR: P01222). Nucleotide numbering starts from the adenine (+1) of the translation initiation codon (ATG) of the NCBI reference sequence NM\_000549.5 and includes the 20-amino acid signal peptide. The seat-belt region is labelled and describes a region between cysteine-108 and cysteine-125 (13).



of Medical Genetics and Genomics guidelines (8), on the basis that the variant is absent from population databases (PM2), detected in *trans* with a pathogenic variant for a recessive disorder (PM3), multiple lines of computational evidence support a deleterious effect (PP3), and the patient phenotype is highly specific for the disease (PP4).

This case demonstrates the importance of strict adherence to thyroxine therapy in CCH associated with pathogenic *TSHB* variants, not only to treat hypothyroidism but also to prevent thyrotrope hyperplasia. Pituitary hyperplasia is a known sequela of untreated primary hypothyroidism, with excessive hypothalamic TRH production stimulating thyrotrope hyperplasia and possible lactotroph hyperplasia (9). Furthermore, radiologically evident pituitary hyperplasia has been described as a hallmark of CCH in patients with *TSHB* pathogenic variants, presumably due to excessive TRH concentrations (10). Unlike primary hypothyroidism, however, TSH levels cannot be accurately used to assess the adequacy of thyroxine replacement in secondary hypothyroidism due to impaired TSH production, as we demonstrate in this case. Importantly, studies have suggested that patients with secondary hypothyroidism are at higher risk of thyroxine under-replacement compared to those with primary hypothyroidism (11). To provide adequate levothyroxine, current guidelines suggest that free T4 levels should be maintained in the middle-to-upper range of normal in patients with central hypothyroidism (12).

This case describes compound heterozygous *TSHB* variants, including the novel c.217A>C (p.(Thr73Pro)), causing CCH, with variable adherence to thyroxine replacement resulting in recurrent pituitary hyperplasia. This case highlights that adequate thyroxine replacement is essential to prevent thyrotrope hyperplasia in patients with CCH, even after pituitary surgery.

#### Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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#### Patient consent

Written informed consent for publication of their clinical details and clinical images was obtained from the patient.

#### Author contribution statement

Adam I Kaplan – Final year medical student; completed the literature research; writing of this report; and preparation of most figures. Catherine Luxford – Laboratory scientist; involved in sequencing and interpretation of the variants; preparation of Fig. 2; preparation of *in silico* results. Roderick J Clifton-Bligh – Adult Endocrinologist; Clinical and laboratory research lead; design of this case report; current Endocrinologist for the patient; expert advisor; involvement in the laboratory sequencing of the variants; editing for this case report.

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