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# Identification of a Novel *IGSF1* Variant in Two Malaysian Male Siblings with Central Hypothyroidism and Macroorchidism

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## What is already known on this topic?

*Immunoglobulin superfamily member 1 (IGSF1)* mutation is the most common cause of X-linked recessive mild to moderate isolated central hypothyroidism. It is associated with macro-orchidism with delayed pubertal testosterone rise, high birth weight, increased body mass index, low prolactin and transient growth hormone deficiency.

## What this study adds?

A novel *IGSF1* c.3467T>A variant was found in two siblings with central hypothyroidism accompanied by macro-orchidism, the first report from South East Asia. Genetic evaluation for *IGSF1* variants is important in patients with unexplained isolated central hypothyroidism +/- macro-orchidism to enable early detection and treatment of hypothyroidism in the proband and other similarly affected family members.

## ABSTRACT

*Immunoglobulin superfamily member 1 (IGSF1)* mutation is the commonest cause of mild to moderate isolated central congenital hypothyroidism and has an X-linked recessive inheritance, primarily affecting males. Other notable clinical features are macro-orchidism with delayed pubertal testosterone rise, large birth weight, increased body mass index, low prolactin and transient growth hormone deficiency. Two male siblings with central hypothyroidism were found to have a novel *IGSF1* c.3467T>A variant that was likely pathogenic based on the family segregation study. The proband, aged 3 years, presented at 18 days old with prolonged jaundice while his 16-year-old brother was only shown to have central hypothyroidism after the genetic analysis result of the proband was known. Both siblings were obese, had large birth

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weights, macro-orchidism and low prolactin. The proband's brother had intellectual disability while the proband had normal development. This case study highlights the importance of evaluation for *IGSF1* variants in patients with unexplained central hypothyroidism, especially when accompanied by X-linked inheritance and macro-orchidism. Family segregation analysis will facilitate detection of other affected family members or carriers who may also benefit from thyroxine treatment.

**Keywords:** *IGSF1* variant, central hypothyroidism, macroorchidism

## Introduction

Central congenital hypothyroidism (CCH) is a thyroid disorder that is not readily detected by thyroid stimulating hormone (TSH)-based neonatal screening programmes due to low levels of both TSH and free thyroxine hormone (FT4). CCH is caused by a mutation in transcription factor genes that mediate pituitary gland development in most (60%) cases, including *POU1F1*, *PROP1*, and *HESX1*, resulting in multiple pituitary hormone deficiencies (1,2). Isolated CCH is rare and can be due to genetic defects in the  $\beta$ -subunit of TSH and thyrotropin-releasing hormone (TRH) receptor (1,2,3). More recently, three other genes have been implicated in isolated central CH, namely, *IGSF1*, *TBL1X* and *IRS4*, all of which are of X-linked inheritance (1).

The *IGSF1* gene resides on X-chromosome (Xq26.2) and is expressed in the pituitary gland, hypothalamus, and testes (1,2,4). It encodes a plasma membrane immunoglobulin superfamily glycoprotein that may be involved in TRH receptor expression in the pituitary gland, and regulates TSH secretion via TRH signaling (1,2). Loss-of-function mutations in *IGSF1* cause TSH deficiency and X-linked recessive mild to moderate central hypothyroidism (OMIM: #300888), primarily affecting males. Other reported clinical features are macro-orchidism with delayed pubertal testosterone rise, delayed adrenarche, low prolactin, large birth weight and obesity (2,5). TSH and prolactin response to TRH stimulation is normal or reduced (1). The phenotype of carrier females ranges from being asymptomatic to having minor manifestations (5).

The *TBL1X* protein forms part of the thyroid hormone receptor corepressor complex. *TBL1X* mutation results in hypothalamus and pituitary gland resistance to low FT4 levels and a negative shift of the FT4 setpoint (1). TSH and prolactin response to TRH test is however normal (1). Hemizygous males with *TBL1X* pathogenic variants have mild to moderate hypothyroidism and some affected individuals also have hearing deficits (1). Patients with *IRS4* gene mutation have isolated CCH which is only mild with a blunted TSH response and normal/slightly low prolactin response to TRH (1). Individuals with *TBL1X* and *IRS4* pathogenic variants do not exhibit the other clinical features of *IGSF1* variants, such as macro-orchidism with delayed adrenarche or large birth weight. As with *IGSF1* mutation, heterozygous females with *TBL1X* and *IRS4* mutations are usually asymptomatic and have low-normal FT4 levels (1).

## Case Presentation

A 3-year-old boy was diagnosed with central hypothyroidism at 18 days old during a workup for prolonged jaundice. He was the sixth child of non-consanguineous parents. His newborn screening cord TSH was 3.997 mIU/L (normal). He was a term infant with a birth weight of 4.3 kg born to a mother with gestational diabetes. He required five days of invasive respiratory support at birth for respiratory distress syndrome. He was later readmitted at 18 days old for nosocomial pneumonia and was found to have prolonged jaundice. A thyroid function test (TFT) performed at that time revealed a normal TSH of 4.3 mIU/L (1.7-9.1 mIU/L) and a low normal FT4 of 10.8 pmol/L (10.5-30 pmol/L). Serial monitoring of thyroid function showed a declining trend in FT4 down to 7.8 pmol/L, with TSH 3.5 mIU/L at 48 days old. He was commenced on L-thyroxine 4 mcg/kg/day daily at two months of age. His thyroxine dose was gradually weaned down from five months of age as his TSH was very low (<0.5 mIU/L) with FT4 levels at the upper range of normal. Thyroxine was later stopped at one year of age due to low thyroxine requirement (1 mcg/kg/day). However, it was restarted one month later as FT4 fell to 9.4 pmol/L (10-17.6 pmol/L) with a lack of TSH response (TSH 5.08 mIU/L) without thyroxine replacement, suggesting central hypothyroidism.

His developmental milestones were normal. Serial growth monitoring revealed weight following the 97<sup>th</sup> percentile since infancy while height was on the 50<sup>th</sup> percentile. His calorie intake was excessive for his age and consisted of rice/noodles with meat and vegetables for his three main meals with three servings of snacks (biscuits/bread/ fresh milk) in a day. His present height, weight and body mass index (BMI) at 3 years of age are 93.9 cm (-0.28 SD), 17.8 kg (+1.82 SD) and 20.2 kg/m<sup>2</sup> (+2.71 SD) respectively. Bilateral testicular enlargement ( $\geq$ 4 mL) was observed as early as 2.2 years of age. The right testis increased to 10 mL while the left enlarged to 6 mL at 3 years of age. There were no other signs of puberty. He had no midline defects or other system abnormalities. His hormonal profile was prepubertal with unstimulated luteinizing hormone (LH) of <0.12 IU/L, follicle stimulating hormone (FSH) 2.21 IU/L and serum testosterone <0.45 nmol/L. His serum prolactin was low 57.2 mIU/L (72-592 mIU/L). Peak cortisol was 656.8 nmol/L (normal >500 nmol/L) post synacthen test. Genetic testing by whole-exome sequencing by a commercial diagnostic genetic laboratory (3billion, South Korea), identified a novel hemizygous

missense variant c.3467T>A (p.Val1156Glu) in the *IGSF1* gene (NM\_001555.5) of uncertain significance.

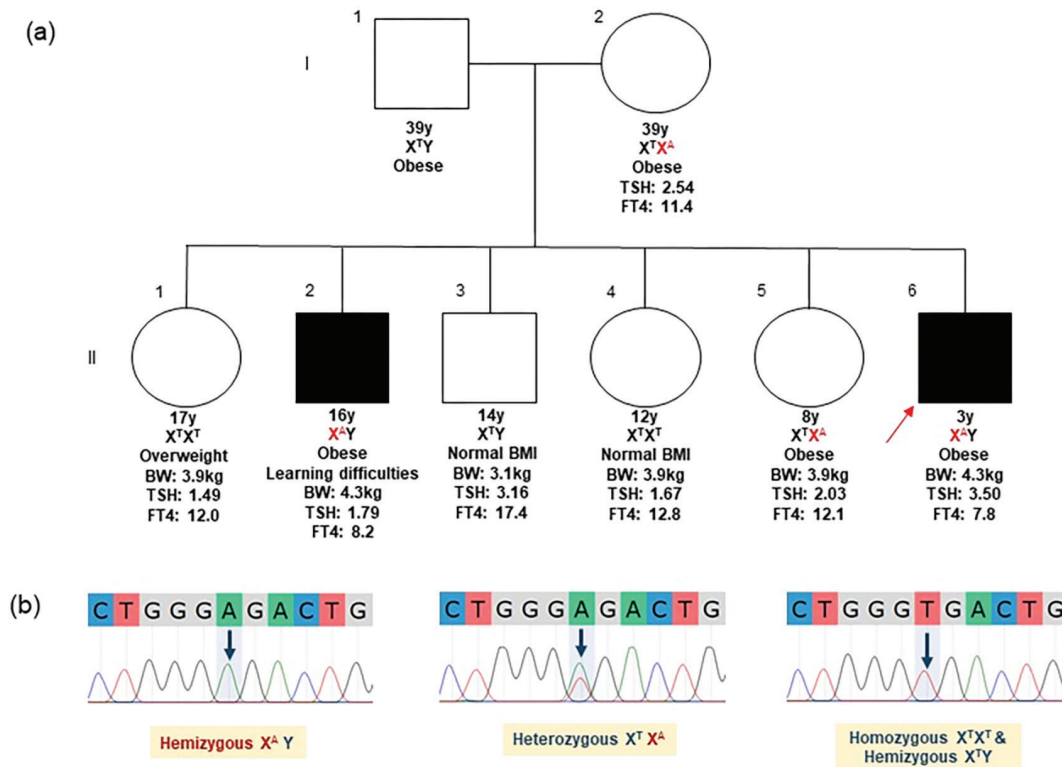
### Family Segregation Study

Seven family members (the proband's parents and five siblings) consented to genetic testing. Blood samples were taken for DNA extraction for targeted Sanger sequencing (by 1st BASE, Malaysia) and screening TFT. The genotype and phenotype of the family pedigree are presented in Figure 1.

The proband's 16-year-old elder brother (II2) was found to carry the same c.3467T>A variant (Figure 1). His screening TFT revealed central hypothyroidism. Like the proband, he was relatively large at birth and had normal newborn cord blood TSH screening. He had speech delay and psychomotor retardation since preschool age. He was a slow learner and had poor social interaction with others. His present height, weight and BMI are 168.8 cm (-0.62 SD), 100.4 kg (+2.39 SD) and 35.2 kg/m<sup>2</sup> (+2.43 SD) respectively at 16 years of age. His Tanner puberty staging was genitalia 4, public hair 3, and both testes were enlarged (>25 mL). He had normal pubertal levels of LH (2.13 IU/L), FSH (9.67 IU/L), and serum testosterone (16.64 nmol/L).

Serum prolactin was also low at <17.22 mIU/L (72-592 mIU/L). His fasting lipid profile and fasting blood glucose were normal. He was commenced on L-thyroxine 1 mcg/kg/day daily upon diagnosis of central hypothyroidism. Since then, he has shown improvement in his mental processing and social functioning with normalization of FT4.

The proband's mother (I2) and 8-year-old sister (II5) who were carriers for the same *IGSF1* variant, c.3467T>A, had normal TFT but were also obese (BMI 35.9 kg/m<sup>2</sup> and 21.5 kg/m<sup>2</sup> (+1.74 SD), respectively). Other siblings (II1, II3, II4) who did not carry the *IGSF1* variant had normal TFT but variable weight status. His 17-year-old sister (II1) was overweight (BMI 28.5 kg/m<sup>2</sup>), while his 14-year-old brother (II3) and 12-year-old sister (II4) had normal BMI. The proband's unaffected siblings had lower birth weights, ranging from 3.0-3.9 kg. The detected *IGSF1* variant (c.3467T>A) was reclassified as likely pathogenic based on the American College of Medical Genetics and Genomics criteria and cosegregation data interpretation in pathogenicity classification (Table 1) (6,7).



**Figure 1.** (a) Family pedigree. Filled black symbols represent individuals affected by central hypothyroidism. The present age in years (y) is shown below the symbols. The genotype is shown below the present age. BW, birth weight; BMI, body mass index; TSH, thyroid stimulating hormone (mIU/L); FT4, free thyroxine (pmol/L). A red arrow indicates the proband. (b) Representative chromatograms for targeted Sanger sequencing of the *IGSF1* gene variants identified in the family.

**Table 1. *IGSF1* (c.3467T>A) classification according to American College of Medical Genetics and Genomics criteria**

Evidence	Category	Description
PM2	Moderate	Absent from controls (or at extremely low frequency if recessive) in Exome Sequencing Project, 1000 Genomes Project, or Exome Aggregation Consortium.
PP1	Strong	Cosegregation with disease in multiple affected family members in a gene definitively known to cause the disease.

Classification: Likely pathogenic by fulling American College of Medical Genetics and Genomics criteria

## Discussion

The first cases of *IGSF1* variant were reported among 11 unrelated families in 2012 who exhibited central hypothyroidism, testicular enlargement, and prolactin deficiency (8). Hitherto, this was the most common cause of mild to moderate isolated CCH among males and females and has an incidence rate of approximately 1:100000 (1,9). Patients with *IGSF1* variants have been reported to express a broad spectrum of clinical manifestations (5,10,11). Central hypothyroidism of variable severity is the main finding in all males with *IGSF1* variant, presenting with symptoms of hypothyroidism at different stages in life (5,10,11). The proband (II6) had mild to moderate CCH when he presented in early infancy with prolonged jaundice. The proband's brother (II2) had a later presentation with speech delay and psychomotor retardation at preschool age, and was only found to have moderate CCH in his teens.

Both siblings share the classical phenotype of the *IGSF1* variant-induced syndrome reported in the literature, including increased birth weight, obesity, macro-orchidism and prolactin deficiency (5). Other studies have reported these patients to be overweight or obese despite thyroid hormone replacement (5,12). The mechanism of increased birth weight and obesity is unknown. The relatively high FSH levels in the proband and untreated hypothyroidism in the affected brother may have contributed to the macro-orchidism (13). As *IGSF1* is also expressed in the testes, it is postulated that loss-of-function mutations in *IGSF1* cause the testicular enlargement (13).

Hyperprolactinemia associated with *IGSF1* mutation is yet to be understood but it can affect adrenal function (13). Prolactin receptors are expressed in the adrenal gland and work synergistically with adrenocorticotropin hormone (ACTH) to augment adrenal androgen secretion (14). Delayed adrenarche is a finding often associated with *IGSF1* mutation with prolactin deficiency (5). However, the pituitary-adrenal axis in *IGSF1* mutation is usually intact with adequate cortisol response on ACTH stimulation test, as shown by the proband (5). The affected brother (II2) did not have an ACTH stimulation test but he had no history to suggest adrenal insufficiency. Fertility has reportedly

been preserved in individuals with *IGSF1* mutations (5). Clinical and biochemical monitoring for adrenarche and puberty would be required for the proband.

The degree of central hypothyroidism varies in individuals with *IGSF1* mutation, and it is unclear at what FT4 levels patients are affected by hypothyroidism. While some untreated adults generally have normal cognitive functioning with normal height, children with prolonged jaundice, obesity, dyslipidemia, and poor growth respond to the initiation of thyroxine therapy (5). In the case of II2, improvement in mental processing and social functioning was observed after thyroxine replacement as per parental report. As for the proband, his normal development is likely attributed to the early initiation of treatment. It is recommended that treatment be started in all male children with *IGSF1* variant and a treatment trial be given to all male adults and female carriers with low FT4 concentrations (5).

## Conclusion

This case study describes the phenotype of two male siblings with a novel *IGSF1* variant, c.3467T>A, that is likely pathogenic based on the family segregation study. The report highlights the importance of genetic testing for *IGSF1* variants in patients with unexplained central hypothyroidism, especially when X-linked inheritance, macro-orchidism without pubarche, high birth weight, obesity or prolactin deficiency are present. Furthermore, a detected *IGSF1* variant on genetic testing of a proband should prompt screening of other seemingly asymptomatic family members who may also benefit from thyroxine replacement.

### Ethics

**Informed Consent:** The proband's family have given written consent for the family segregation study and to publish their case.

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## Footnotes

### Authorship Contributions

Surgical and Medical Practices: Yee Lin Lee, Tzer Hwu Ting, Concept: Yee Lin Lee, Tzer Hwu Ting, King Hwa Ling, Design: Yee Lin Lee, Data Collection or Processing: Yee Lin Lee, Chong Teik Lim, Karuppiyah Thilakavathy, Nurul Huda Musa, King Hwa Ling, Analysis or Interpretation: Yee Lin Lee, Tzer Hwu Ting, Chong Teik Lim, Karuppiyah Thilakavathy, Nurul Huda Musa, King Hwa Ling, Literature Search: Yee Lin Lee, Tzer Hwu Ting, Writing: Yee Lin Lee, Tzer Hwu Ting, Chong Teik Lim, Karuppiyah Thilakavathy, King Hwa Ling.

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