

## Congenital Early Onset Isolated Adrenocorticotropin Deficiency Associated with a *TPIT* Gene Mutation

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### ABSTRACT

**TPIT is a highly cell-restricted transcription factor that is required for the expression of the proopiomelanocortin (POMC) gene and for terminal differentiation of the pituitary corticotroph lineage. Its exclusive expression in pituitary POMC-expressing cells has suggested that its mutation may cause isolated deficiency of pituitary ACTH. We present a neonate with the diagnosis of congenital early onset isolated ACTH deficiency (IAD) associated with a loss of POMC function as a result of a missense mutation in the *TPIT* gene. A 5 day-old male infant was admitted for hypoglycemia, limpness and conjugated hyperbilirubinemia. Laboratory investigations indicated low plasma cortisol concentration (0.1 µg/dl) accompanying a very low ACTH (<5 pg/ml) concentration. An increase in plasma cortisol concentration following stimulation with low dose exogenous ACTH was observed. On replacement therapy with hydrocortisone (15 mg/m<sup>2</sup>/day orally), cholestatic jaundice and hypoglycemia resolved and subsequent normal growth (weight, height and head circumference, 25<sup>th</sup>, 10<sup>th</sup> and 50<sup>th</sup> percentile, respectively) and development was achieved without recurrence of hypoglycemic episodes.**

### KEY WORDS

isolated ACTH deficiency, POMC, *TPIT* mutation, hypoglycemia, cholestasis, neonate

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### INTRODUCTION

During organogenesis, specification of pituitary cell types has been shown to result from several signal-dependent transcription factors. The identification of different proteins involved in differentiation pathway of pituitary cell type and analysis of the expression patterns provide information for developmental defects<sup>1</sup>. Mutation in *TPIT* - a T box factor cooperating with *Pitx1* homeoproteins that is required for proopiomelanocortin (POMC) gene expression - was first identified by Lamolet *et al.* in 2001<sup>2</sup>. POMC is processed in the anterior hypophysis to adrenocorticotropin (ACTH) and  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ -MSH). In most instances ACTH deficiency is diagnosed as part of combined pituitary hormone deficiency. Congenital isolated ACTH deficiency is very rare. Clinical findings present early in the perinatal period. It is a rare cause of neonatal hypoglycemia and convulsions and may arise in association with a prior birth trauma<sup>3</sup>.

We describe a neonate in whom the early onset of isolated ACTH deficiency with an unidentifiable cause was later associated with a missense *TPIT* gene mutation.

### PATIENT REPORT

The patient was the male product of a term pregnancy of a 40 year-old mother. His birth weight was 3,200 g. He was delivered after an uncomplicated vaginal delivery with APGAR scores of 7 and 9 at the 1<sup>st</sup> and the 5<sup>th</sup> minutes, respectively. The infant was admitted to hospital with signs of jaundice, poor feeding and hypoactivity on the 5<sup>th</sup> day of life. His initial physical findings were as follows: body weight 3,430 g, head circum-

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ference 34 cm, pulse 116/min, blood pressure 60/30 mm Hg, respiratory rate 31/min and body temperature 36.6°C. The jaundiced infant was hypotonic and hypoactive; sucking and Moro reflexes were diminished. No facial abnormality was noted. He had hyperemia around the umbilicus. The stretched penile length was 3 cm and his testicles were palpable in the scrotum. The laboratory findings yielded a normal complete blood count with normal white cell differential, urinalysis, calcium and electrolyte values. Blood glucose was 16 mg/dl. Apart from an umbilical swab culture revealing staphylococci, blood, cerebrospinal fluid and urine cultures were negative. Total and direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP),  $\gamma$ -glutamyl transpeptidase (GGT) levels were 17 and 3.2 mg/dl, 38 IU/l, 36 IU/l, 342 IU/l, 102 IU/l, respectively. Etiology of the cholestatic jaundice was investigated. Urine reducing substance, urine and blood amino acids, and viral serology were negative. Sweat test and serum  $\alpha$ -1 antitrypsin level were also within normal limits. Arterial blood gases and serum ammonia level were within the normal range. Abdominal ultrasound and hepatobiliary

scans were unrevealing. Liver biopsy was not performed. Cranial tomography failed to reveal any anatomical abnormality of the sella or suprasellar area. Results of hormone levels are summarized in Table 1. Low ACTH and cortisol levels were detected. Cortisol replacement therapy was started immediately after the assessment of cortisol secretion with the stimulation of exogenous ACTH (1  $\mu$ g synthetic human corticotropin). Hypoglycemia was corrected with glucose infusion and cortisol therapy. Cholestatic jaundice also disappeared in the second week of hydrocortisone therapy.

There was consanguinity between the parents and a congenital genetic defect was suspected as no other cause could be identified. The *TPIT* gene sequence in the patient and family members was investigated for mutations within the coding exons of the gene at Laboratoire de Genetique Moleculaire, GlaxoWellcome, Institut de Recherches Cliniques de Montreal, Canada. As described in the article by Pulichino *et al.*<sup>4</sup>, all eight exons of the *TPIT* gene were amplified from genomic DNA extracted from peripheral lymphocytes by use of eight sets of flanking intronic primers for direct

TABLE I  
Endocrinological findings

	Patient	Normal values
ACTH (pg/ml)	<5	5-50
Plasma cortisol ( $\mu$ g/dl)	0.1	2-10
Plasma cortisol ( $\mu$ g/dl) (after low dose ACTH stimulation)	10	>20
Prolactin (ng/ml)	8.6	2.7-17.9
Thyrotropin stimulating hormone (mU/ml)	4.43	0.465-4.68
Total thyroxine ( $\mu$ g/dl)	7.6	71.2-142
Free thyroxine (pmol/l)	11.3	10-28.2
Total triiodothyronine (nmol/l)	3.19	1.49-2.60
Free triiodothyronine (pmol/l)	6.73	4.26-8.10
Plasma osmolarity (mOsm/kg)	290	275-290
Urine osmolality (mOsm/kg)	340	300-1200

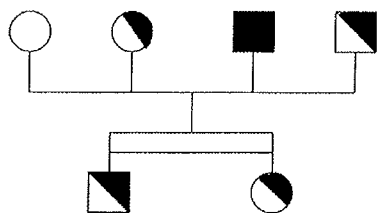


Fig. 1: Pedigree of the patient. The black symbol represents the proband. All family members except one sister are heterozygous for the mutation. The double horizontal bars represent consanguinity.

sequencing. After amplification, PCR products were purified on agarose gel and a CEQ 2000 sequencer (Beckman-Coulter) was used. Cell culture, transfection and plasmids, gel retardation assays and Western blotting were performed as previously described<sup>4</sup>.

The infant was homozygous for a missense mutation in the *TPIT* gene that changes an amino acid in the T box: I171T. Analysis of DNA of other family members indicated that they were all heterozygous carriers of the mutation and were all unaffected (Fig. 1). Currently, the patient is 21 months old and on cortisol treatment at a dose of 10 mg/m<sup>2</sup>/day orally and has normal growth (weight, height and head circumference 25<sup>th</sup>, 10<sup>th</sup> and 50<sup>th</sup> percentiles, respectively) and development.

#### DISCUSSION

Hypoglycemia associated with an endocrine deficiency is usually due to adrenal insufficiency together with or without growth hormone deficiency<sup>5</sup>. The etiology of hypoglycemia in cortisol deficiency may either be due to decreased gluconeogenic enzymes or failure to supply endogenous gluconeogenic substrate in the form of alanine and lactate with compensatory breakdown of fat and generation of ketones<sup>6</sup>. The endocrinological evaluation of the infant revealed low basal cortisol and ACTH levels which may be the reason for the unexplained hypoglycemia and cholestatic jaundice. The patient had findings more consistent with ACTH deficiency than with primary adrenocortical insufficiency. The infant was euthyroid. Baseline gonadotropin levels were within normal limits of

neonatal stage. Furthermore, normal stretched penile length and normal testicular location were considered evidence of intact hypothalamo-pituitary-gonadal axis<sup>7</sup>. Prolactin level was within the normal range. Although baseline growth hormone level was low for neonatal stage, it should be followed up at an older age before making the diagnosis of growth hormone deficiency. In the literature, three children with isolated ACTH deficiency were thought to have an acquired traumatic defect in neuro-endocrine regulation with the disorder in corticotrophin releasing factor leading to secondary adrenal insufficiency. Each of these cases was the product of a complicated pregnancy or delivery<sup>8</sup>. Our patient had no history of mechanical or asphyxiating birth injury and seemed not to be associated with any kind of acquired insult.

Congenital IAD is very rare. A few cases have been described with onset from the perinatal period to early adolescence<sup>4</sup>. Both the pathophysiology and clinical description of this condition are poorly defined. Although corticotrophin releasing hormone or its receptor gene have been proposed as candidates for this condition, *TPIT* is the first gene to exhibit a specificity of expression consistent with IAD<sup>4</sup>. It has been demonstrated with a *Tpit*-deficient mouse model that terminal differentiation to corticotrophs and melanotrophs cannot be achieved in spite of well-formed precursors of POMC cells in the absence of this transcription factor which acts on the POMC gene promoter. Because of its highly restricted expression, *Tpit* deficiency may only affect pituitary POMC production<sup>2</sup>. Pulichino *et al.*<sup>4</sup> measured plasma ACTH levels in *Tpit* mutant mice who were homozygous (-/-), heterozygous (-/+) and wild type (+/+). Only in the homozygous mutant mice were adrenal glands hypoplastic and plasma ACTH and corticosterone levels low. This mouse model supports the role of POMC-derived peptides in the maintenance of adrenal tissue. Clinically these mutant mice had seizures and fasting hypoglycemia. Pulichino *et al.*<sup>4</sup> report the physiological phenotype of *Tpit* null mice as a model of human IAD, and provide a genetic analysis of the first series of human patients with congenital IAD, including the present patient. These studies define a

separate, very homogeneous and previously unrecognized clinical profile of early onset IAD that is associated at high frequency with mutations in the *TPIT* gene (8/11 patients investigated). The characterization of seven different loss-of-function *TPIT* mutations (I171T in our patient) offers a molecular explanation for this recessive disease.

Data in the present report show that jaundice is associated with cortisol insufficiency. Hypopituitarism associated with liver dysfunction was previously reported in 13 patients. Twelve had evidence of hypoglycemia, and cortisol insufficiency was present in nine of them<sup>9,10</sup>. There is experimental evidence that cortisol can influence bile formation. A reduction of bile flow has been observed in animals after adrenalectomy<sup>11</sup>.

Hypoglycemia in a neonate with the above-mentioned clinical and laboratory findings should prompt adrenal function testing. Presence of liver dysfunction can be an additional clue to adrenal insufficiency. Cortisol deficiency, primary or secondary to ACTH deficiency, is among the etiological factors of neonatal hypoglycemia and cholestasis. When ACTH deficiency is diagnosed not associated with any other hormone deficiency, and if the etiology cannot be identified, one should suspect a hereditary condition. The diagnosis of the disorder is important because it is easily correctable but potentially fatal.

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