CLINICAL STUDY

TPIT mutations are associated with early-onset, but not late-onset isolated ACTH deficiencyL A Metherell¹, M O Savage¹, M Dattani², J Walker³, P E Clayton⁴, I S Farooqi⁵ and A J L Clark¹¹Department of Endocrinology, Barts and the London, Queen Mary University of London, London, UK, ²BEM Unit, Institute of Child Health, London, UK, ³Department of Paediatrics, St Mary's Hospital, Portsmouth, UK, ⁴Department of Endocrinology, Royal Manchester Children's Hospital, Manchester, UK and ⁵University of Cambridge Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge, UK

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Abstract

Objective: Congenital isolated ACTH deficiency (IAD) is a rare inherited disorder that is clinically and genetically heterogeneous. Patients are characterised by low or absent cortisol production secondary to low plasma ACTH despite normal secretion of other pituitary hormones and the absence of structural pituitary defects. Onset may occur in the neonatal period, but may first be observed in later childhood. Recently, mutations in the *TPIT* gene, a T-box factor selectively expressed in developing corticotroph cells, have been found in cases of early-onset IAD.

Design: Here we report the screening of the *TPIT* gene in seven patients with IAD, four of whom had neonatal onset.

Methods: Genomic DNA was extracted and the sequences of the 8 *TPIT* exons and their intron/exon junctions were determined by automated sequencing.

Results: Two siblings with early-onset IAD were both compound heterozygotes for mutations in exons 2 and 6. The missense mutation (Met86Arg) in exon 2 within the T-box (or DNA binding domain) is predicted to disrupt DNA binding. A frameshift mutation in exon 6 (782delA) introduces a premature stop codon and is likely to lead to a non-functional truncated protein. No nucleotide changes were observed in exonic sequences in the other two early- or the three later-onset cases. Fifteen single nucleotide polymorphisms that were not predicted to change the *TPIT* transcript were also detected.

Conclusions: These findings provide a further illustration of the genetic heterogeneity of IAD and are highly suggestive of one or more other genes being implicated in this disorder.

European Journal of Endocrinology 151 463–465**Introduction**

Congenital isolated adrenocorticotrophin (ACTH) deficiency (IAD) is a rare inherited disorder that is clinically and genetically heterogeneous. Patients are characterised by low or absent cortisol production secondary to low plasma ACTH despite normal secretory indices of other pituitary hormones and the absence of structural pituitary defects. When tested, there is often no ACTH response to exogenous corticotrophin releasing hormone (CRH). Onset may occur in the neonatal period, but may first be observed in later childhood (Table 1). On stimulation by CRH, ACTH is produced from the ACTH precursor proopiomelanocortin (POMC) by the action of the cleavage enzyme prohormone convertase 1 (PC1). Hence candidate genes for ACTH deficiency include *POMC*, *PC1*, *CRH* and its receptor (*CRH-R1*). However, mutations in these genes have not been reported to be associated with IAD. Indeed *POMC* gene defects have been shown to

result in a phenotype of early-onset ACTH deficiency combined with red hair and obesity (1). *PC1* mutations have been described in a woman with extreme childhood obesity, abnormal glucose homeostasis and both ACTH and gonadotrophin deficiency (2) and also in a girl with ACTH deficiency, obesity and gastrointestinal abnormalities (3). Although linkage of the *CRH* gene locus to IAD has been observed in one family (4), no mutations in this gene or its receptor have been described in humans. This implies that other genes/pathways must be involved in this disease. The differentiation of pituitary cell types during embryonic development is regulated by the combined action of a subset of tissue- and cell-restricted transcription factors. Two such factors have been reported in the *POMC*-expressing lineages of the pituitary, *NeuroD1* and *TPIT*. *NeuroD1* contributes to cell-specific transcription of the *POMC* gene in corticotroph cells and is required for early corticotroph differentiation (5). Defects in *NeuroD1* are a cause of maturity onset diabetes of the

Table 1 Clinical and biochemical details of the IAD patients.

Normal range	Early-onset IAD				Later-onset IAD		
Patient number	1	2	3	4	5	6	7
Sex	M	F	M	F	M	F	M
Age of onset	8 months	1 hour	36 hours	Infancy	7 years	8 years	3 days
Basal cortisol (nmol/l)	12	<13	109	130	undetectable	<50	44
Plasma ACTH (ng/l)	10	n.d.	undetectable	<5	<5	undetectable	35
Short synacthen test	12	72	237	270	<28	<50	621
peak cortisol (nmol/l)							
Long synacthen test	66	127	n.d.	n.d.	885	890	n.d.
peak cortisol (nmol/l)							
CRF test	n.d.	n.d.	n.d.	ACTH <5 cortisol <20	ACTH <5	ACTH <2.2 cortisol <50	ACTH 6–18 cortisol <25

CRF test, corticotropin releasing factor test; n.d., not determined. Units for ACTH and cortisol levels in the CRF test are ng/l and nmol/l respectively.

young (6). *TPIT*, a recently described transcription factor, is required for the terminal differentiation of corticotroph cells. Recently, mutations in the *TPIT* gene have been reported in cases of early onset IAD (7–9) (Fig. 1). *TPIT* is a member of the T-box gene family, the defining feature of which is a region of conserved DNA sequence (the T-box) which encodes a DNA binding domain (10). We report the results of analysis of *TPIT* in seven patients with IAD.

Subjects and methods

Subjects

Seven patients with IAD, four with early onset (<1 year of age) and three with later onset (>5 years of age) (Table 1) were studied. These patients belong to six unrelated families (patients 1 and 2 are siblings) and include a patient (no. 6) previously described by us (11). Criteria for inclusion in this patient cohort were low or absent cortisol production with normal levels of other pituitary hormones and an absence of structural pituitary defects.

Clinical investigations

Clinical details and outcomes of endocrine investigations are summarised in Table 1.

Sequencing

The sequences of the eight coding exons and their splice junctions were determined by PCR and automated sequencing utilising primers derived from intronic sequences of the *TPIT* gene (PCR primers and conditions on request).

Mismatch PCR for M86R mutation

Two PCRs were performed on each normal DNA sample utilising either a wild-type (GGGTTGGACCCCAATGCCAT) or mutant (GTTGGACCCCAATGCCAG) forward primer and a common reverse primer (CTCCCATCTGTGTCTCCAATCC). Parental DNAs were used as positive and negative controls for the mutation (reaction conditions on request).

Figure 1 Summary of human *TPIT* gene mutations found in early onset isolated ACTH deficiency (IAD). Patients 1 and 2 were heterozygous for 782delA and M86R (a novel mutation) indicated in boxes. Mutations indicated with short arrows and the 5.2 kb deletion have been reported elsewhere (see text).

Results

Two mutations were identified in two siblings with early-onset IAD (patients 1 and 2). The first mutation, in exon 2, changes a single amino acid residue (M86R) whilst the other, in exon 6, is a frameshift mutation (782delA) previously reported to be destroyed by nonsense mediated mRNA decay (9). These mutations are shown in Fig. 1 together with other reported mutations. The mother of the affected siblings was heterozygous for the wild-type allele and M86R whilst the father was a heterozygote for wild-type and 782delA alleles. The exon 2 mutation is predicted to result in a non-functional protein due to disruption of DNA binding in the mutant T-box. Mutations were not identified in the other two early-onset or the three later-onset IAD cases and screening of 50 normal subjects by mismatch PCR failed to identify the novel exon 2 mutation. Fifteen single nucleotide polymorphisms (SNPs) were identified within intronic sequences in the regions analysed but none of these SNPs are predicted to cause a change in the *TPIT* transcript or splice variations.

Discussion

The mutations reported to date are mostly clustered in the T-box region of the gene (Fig. 1) and as such might be expected to lead to loss-of-function through a disruption in DNA binding. However, many of the mutant transcripts (R179X, R286X and 782delA), which would be predicted to result in premature truncation of the protein, are reportedly destroyed by nonsense mediated mRNA decay (9). The mRNA transcripts resulting from the 573del4 mutant (8) and the 5.2 kb deletion (9) may be similarly destroyed or lead to severely truncated proteins. In contrast, the mutant T58A, S128F and I171T transcripts produce defective proteins. The S128F and I171T proteins showed no transcriptional activity or DNA binding capacity (9), whereas the T58A mutant protein had greatly reduced levels of both transcription and DNA binding (9). We predict the novel mutant M86R would cause a disruption of DNA binding since this residue is within the T-box region and is conserved in the mouse, human and chicken *TPIT* orthologues.

Conclusions

In agreement with previous studies (9) we found *TPIT* mutations in some (2/4) but not all early-onset cases of IAD and no *TPIT* mutations in patients with late-onset IAD. Furthermore, no phenotypic differences between early-onset IAD cases with and without mutations were apparent. It is feasible that mutations within regulatory regions of the gene could be responsible for some of these cases; however the finding

that no late-onset patients have mutations in *TPIT* is highly suggestive of at least one other gene being involved in this disorder.

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