MISSPLICING DUE TO A SILENT EXONIC SUBSTITUTION IN THE T-BOX TRANSCRIPTION FACTOR TBX19 RESULTING IN ISOLATED ACTH DEFICIENCY

Ashwini Maudhoo[1], Avinaash Maharaj[1], Federica Buonocore[2], Gabriel Angel Martos-Moreno[3], Jesús Argente[3, 4], John Achermann[2], Li Chan[1], Lou Metherell[1]

[1] Centre for Endocrinology, William Harvey Research Institute, Barts and the London School of Medicine and Dentistry, Queen Mary University of London, London, UK; [2] Genetics and Genomic Medicine, UCL Great Ormond Street Institute of Child Health, University College London, London, UK; [3] Department of Endocrinology, Hospital Infantil Universitario Niño Jesús, Instituto de Investigación Sanitaria La Princesa. Universidad Autónoma de Madrid. CIBER obn. Instituto de Salud Carlos III. Madrid, Spain; [4] IMDEA Food Institute. Madrid, Spain.

INTRODUCTION

Congenital isolated ACTH deficiency (IAD) → low plasma ACTH and serum cortisol with preserved function of all other pituitary hormones.

TBX19 is a **T-box transcription factor** involved in the terminal differentiation of pituitary **POMC** expressing cells (Figure 1). Of the 25 *TBX19* mutations associated with IAD, most are missense but 5 have been described to affect splicing.[2]

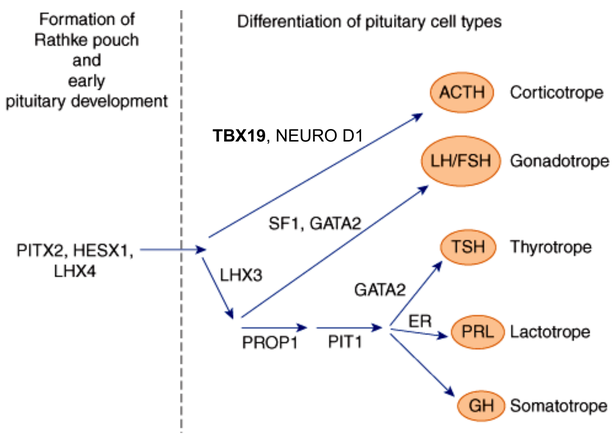

Fig 1. Development of the pituitary gland.[1]

CLINICAL CASE

We report a neonate of Romanian origin, who presented at 15 hours of life with **respiratory arrest and hypoglycaemia**. Over the following 2 weeks **recurrent hypoglycaemia** was documented.

On examination → **normal male genitalia and no hyperpigmentation**
Biochemical investigations:
→ **Undetectable serum cortisol** (cortisol <1 µg/dl; NR 7.8-26.2) and
→ **Inappropriate plasma ACTH levels** (22.1 pg/ml; NR 4.7- 48.8)

He responded to **hydrocortisone treatment** and continues on replacement. He has a healthy sister who is 30 months older. However, there is a family history of adrenal disease as his aunt (mother's sister) has been on hydrocortisone treatment since 18 months of age with a diagnosis of suspected IAD.

METHODS & RESULTS

DNA → analysed by a **HaloPlex next-generation sequencing** array targeting genes for adrenal insufficiency & variants → filtered by **Ingenuity Variant Analysis** (Figure 2).

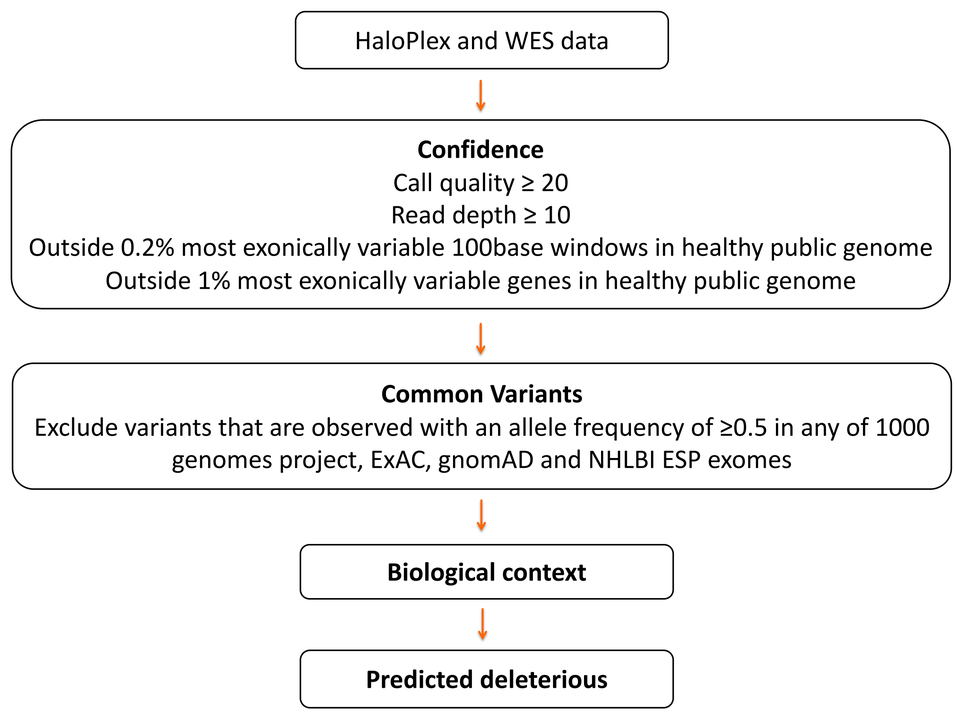

Fig 2. Filtration strategy for variant screening from WES and HaloPlex data (Adapted from Chan *et al.*)[3]

Chromosome	1	**Protein variant**	p.T96=
Position	168260482	**Translation impact**	Synonymous
Reference allele	G	**Genotype**	Homozygous
Sample allele	A	**CADD score**	< 10
Gene region	Exonic	**dbSNP**	rs376493164
Gene symbol	TBX19	**gnomAD frequency (%)**	0.001

Table 1. Details of *TBX19* variant found in the proband.

A **novel, homozygous, extremely rare, synonymous variant p.Thr96=** was found in **exon 2** of the ***TBX19* gene** (Figure 3; Table 1).

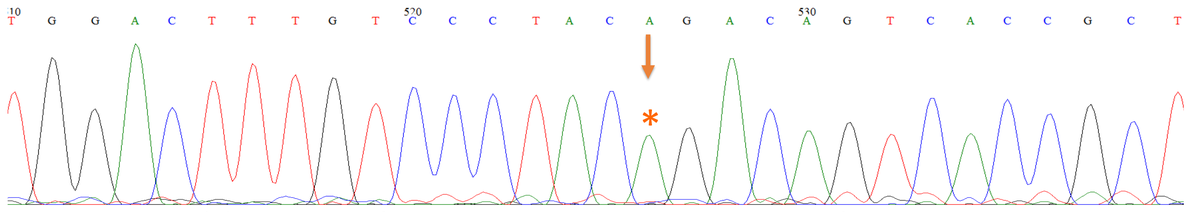

Fig 3. Partial chromatogram of Sanger sequencing showing **c.288G>A p.T96= homozygous variant** present.

Analysis of the variant using the **Human Splicing Finder (HSF)** software **predicted**:
- A **new acceptor site** with a **consensus value of 92.39**
- Loss of 86bp → a frameshift → early stop codon → truncated protein

The effect of the novel mutation was assessed by an ***in vitro* splicing assay**, pET01 ExonTrap cloning vector (MobiTec), comparing wild type and mutant heterologous minigenes.

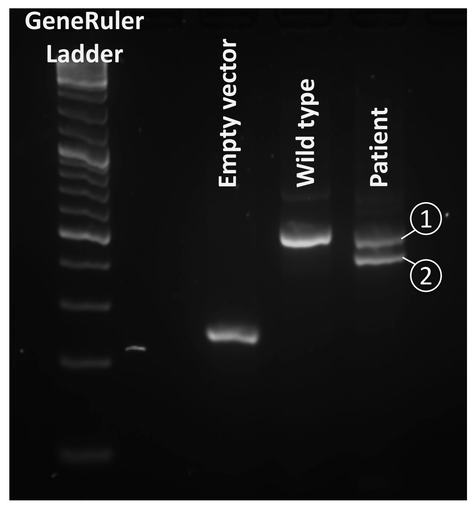

Fig 4a. Gel electrophoresis of PCR products from *TBX19* splicing assay.

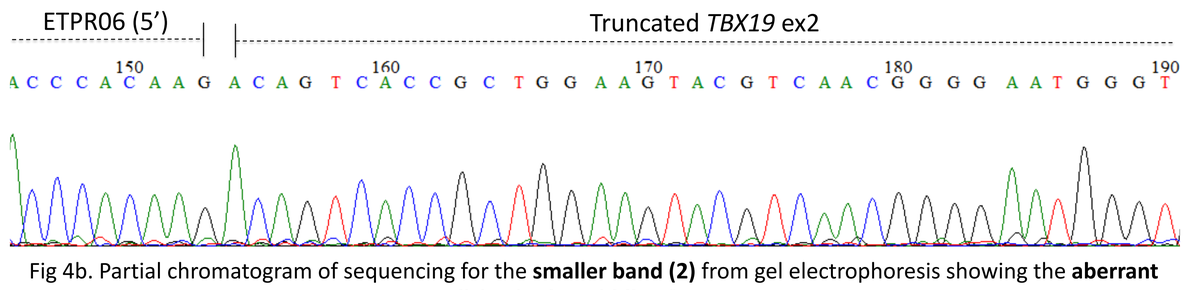

Fig 4b. Partial chromatogram of sequencing for the **smaller band (2)** from gel electrophoresis showing the **aberrant splicing in the middle of exon 2**.

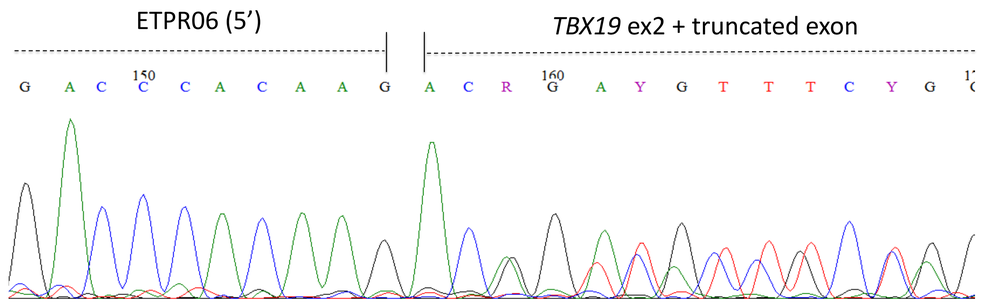

Fig 4c. Partial chromatogram of sequencing for the **larger band (1)** from gel electrophoresis shows sequences for both the **normally spliced exon 2** and **splicing in the middle of exon 2** suggesting a **hybrid of the two sequences**.

The mutation results in **aberrant splicing of exon 2,** giving rise to a mutant mRNA transcript whereas the wild-type vector spliced exon 2 normally (Figure 4a-c, 5).

CONCLUSION

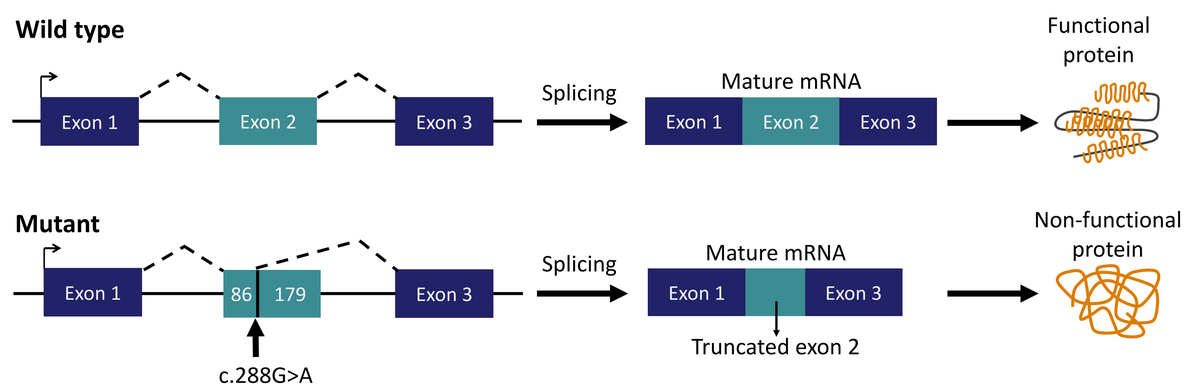

Fig 5. Cartoon of the *TBX19* exon 2 truncation where the c.288g>A variant causes skipping of the first 86bp of exon 2.

We have identified a translationally **silent *TBX19* mutation** causing **aberrant splicing** as the likely cause of isolated ACTH deficiency in the patient. Most mRNA transcripts are aberrantly spliced leading to a **truncated**, **non-functional** protein in keeping with the **complete loss of cortisol production** and **early presentation** in the patient.

References
1. Else T, Hammer GD. *Disorders of the hypothalamus and pituitary gland*. 7th ed. McGraw-Hill Education; 2014.
2. Stenson PD, Ball EV, Mort M, Phillips AD, Shiel JA, Thomas SJ, *et al*. *The Human Gene Mutation Database (HGMD)*. Available from: http://www.hgmd.cf.ac.uk/ac.
3. Chan LF, Campbell DC, Novoselova TV, Clark AJL, Metherell LA. Whole-Exome Sequencing in the Differential Diagnosis of Primary Adrenal Insufficiency in Children. *Frontiers in Endocrinology*. 2015;6:113. doi: 10.3389/fendo.2015.00113.