Pseudoexon activation in nicotinamide nucleotide transhydrogenase (NNT) in two siblings with Familial Glucocorticoid Deficiency

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Introduction

Intronic DNA frequently encodes potential exonic sequences called pseudoexons, i.e. sequences very similar to exons with 5' and 3' splice-sites that are ignored by the cellular splicing machinery and therefore not included into mature mRNA. To date aberrant pseudoexon inclusion has been implicated in approximately 50 genes in various diseases.

Clinical presentation

The proband (Fig. 1A, II:2) presented at 21 months of age, unresponsive with hypoglycaemia (serum blood glucose 0.6 mmol/L (normal range (N.R) 3.0-5.4 mmol/L). Endocrine evaluation subsequent to resuscitation indicated adrenal insufficiency with elevated plasma ACTH 492 pmol/L (N.R 2.2-13.3 pmol/L), low cortisol 30 mmol/L (N.R. 138-655 nmol/L), normal serum aldosterone (192 pmol/L) and appropriately suppressed insulin <2mU/L with ketosis. The child had hyperpigmentation of the skin (Fig. 1B). Hydrocortisone therapy was commenced upon diagnosis of FGD. A sibling (Fig. 1A, II:1), 4 years younger than the proband had a short synacthen test (SST) performed on day 4 of life: baseline cortisol 38 nmol/L with a 60 minute peak of 380 nmol/L. Hydrocortisone was not instituted at that stage as the infant was clinically well and remained under surveillance. Increased pigmentation was noted by her parents from 6 months of age. Following a gastrointestinal illness at 8 months of age a second SST was undertaken; baseline serum cortisol was 110 nmol/L with a peak of 130 nmol/L at 60 minutes, consistent with adrenal insufficiency. Hydrocortisone was commenced immediately.

Fluorochromy was not required for either child. Both siblings have normal thyroid function, Hba1c and fasting glucose and insulin levels.

Objective & Methods

To find the genetic cause of the siblings FGD whole exome sequencing was undertaken on genomic DNA of the siblings. Variants in the seven genes known to cause FGD; M2RP, MRAP, STAR, CYP11A1, NNT, MCP4 and TXNRD2, were assessed for causality. Further analysis of genomic DNA and cDNA was performed by PCR/RT-PCR followed by automated Sanger sequencing.

Results

Whole exome sequencing identified a novel, heterozygous variant g:543613069C>T; c. 211C>T; p. R711* in the antioxidant defence gene NNT, in both affected individuals II:2 and II:5 (Fig. 2A). This heterozygous R711* variant was also found in the DNA of the unaffected sibling (II:1) and their mother (I:2) (Fig. 2A). The pattern of inheritance of FGD is recessive so it was unlikely that, on its own, this heterozygous change was causative especially since it was also carried by the unaffected mother and sister. To determine whether there was an intrinsic lesion on the proband’s other allele cDNA sequencing was undertaken after RNA protection with calf-zeaxanthin. Analysis of the patient’s cDNA revealed the inclusion of a 69bp pseudoexonic sequence from intron 20 of the NNT gene [c.2995_2996insAG_032869] (Figure 2B & C). If translated this would result in a frameshift and creation of a premature stop codon at position 1000, p.D999fs*.2. No such inclusion was seen in the cDNA of the unaffected sibling (II:1) or their mother (I:2) (data not shown). Further analysis of the genomic DNA of the patient identified a 4 bp duplication, g:543701537_437015448dupAGTA, within intron 20 that is predicted by Human Splicing Finder (www.umd.be/HSF3) to create an aberrant splice acceptor site and is presumably responsible for the pseudoexon activation (Figure 2C & D). This change was novel, also identified in the affected sibling (II:5) and was inherited from the father (I:1) who does not have the R711* variant or FGD (2D). Neither of the heterozygous NNT sequence changes identified in this family has been annotated in dbSNP or the NHLBI exome variant server (www.ncbi.nlm.nih.gov/SNP; http://evs.gs.washington.edu/EVS/) and both are predicted to be protein damaging.

Conclusions

The patients reported in this study had a late onset, progressive presentation of their FGD, typical for mutations in genes involved in oxidative stress regulation. Here we report two novel, compound heterozygous mutations in NNT, including one which activates a pseudoexon, as the cause of FGD in the two siblings. Aberrant pseudoexon inclusion is rarely recognised as a cause of human disease, since, for practical reasons, intronic sequences are not usually included in mutational studies. In this case an intronic mutation was suspected because of the clue of one defective NNT allele, however, in consanguineous pedigrees, with a homozygous defect, or in dominant inheritance models this could easily be missed. This case highlights the importance of cDNA analysis to investigate the possibility of non-coding variants contributing to disease.