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Case Report

# Long-term outcome of isobutyryl-CoA dehydrogenase deficiency diagnosed following an episode of ketotic hypoglycaemia

S. Santra [a,*], A. Macdonald [b], M.A. Preece [c], R.K. Olsen [d], B.S. Andresen [d,e]

[a] Department of Clinical Inherited Metabolic Disorders, Birmingham Children's Hospital, Steelhouse Lane, Birmingham B4 6NH, United Kingdom
[b] Department of Dietetics, Birmingham Children's Hospital, Steelhouse Lane, Birmingham B4 6NH, United Kingdom
[c] Department of Newborn Screening and Biochemical Genetics, Birmingham Children's Hospital, Steelhouse Lane, Birmingham B4 6NH, United Kingdom
[d] Research Unit for Molecular Medicine, Department of Clinical Medicine, Aarhus University and Aarhus University Hospital, Aarhus, Denmark
[e] The Villum Center for Bioanalytical Sciences and Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, Denmark

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ABSTRACT

Isobutyryl-CoA Dehydrogenase Deficiency (IBDD) is an inherited disorder of valine metabolism caused by mutations in *ACAD8*. Most reported patients have been diagnosed through newborn screening programmes due to elevated C4-carnitine levels and appear clinically asymptomatic. One reported non-screened patient had dilated cardiomyopathy and anaemia at the age of two years. We report a 13 month old girl diagnosed with IBDD after developing hypoglycaemic encephalopathy (blood glucose 1.9 mmol/l) during an episode of rotavirus-induced gastroenteritis. Metabolic investigations demonstrated an appropriate ketotic response (free fatty acids 2594 μmol/l, 3-hydroxybutyrate 3415 μmol/l), mildly elevated plasma lactate (3.4 mmol/l), increased C4-carnitine on blood spot and plasma acylcarnitine analysis and other metabolic abnormalities secondary to ketosis. After recovery, C4-carnitine remained increased and isobutyrylglycine was detected on urine organic acid analysis. Free carnitine was normal in all acylcarnitine samples. IBDD was confirmed by finding a homozygous c.845C > T substitution in *ACAD8*. The patient was given, but has not used, a glucose polymer emergency regimen and after ten years' follow-up has had no further episodes of hypoglycaemia nor has she developed cardiomyopathy or anaemia. Psychomotor development has been normal to date. Though we suspect IBDD did not contribute to hypoglycaemia in this patient, patients should be followed-up carefully and glucose polymer emergency regimens may be indicated if recurrent episodes of hypoglycaemia occur.



## 1. Introduction

Isobutyryl-CoA Dehydrogenase (IBD) is a mitochondrial enzyme which catalyses the conversion of isobutyryl-CoA to methylacrylyl-CoA in the third step of valine catabolism [1]. It is encoded by *ACAD8* located on chromosome 11q25. Patients with inherited IBD deficiency (IBDD; McKusick 611283) have mostly been diagnosed through newborn screening programmes due to elevated C4-carnitine levels [2–5]. C4-carnitine may represent butyrylcarnitine (as seen in short chain acyl-CoA dehydrogenase deficiency, SCADD) or isobutyrylcarnitine from IBDD. Algorithms have been developed to distinguish these two conditions in newborns with elevated C4-carnitine levels [6]. Patients with IBDD diagnosed in this way have appeared clinically asymptomatic though a few patients have shown speech delay. It is uncertain what treatment, if any, is required following diagnosis. One reported patient who was not diagnosed from a newborn screening programme had anaemia and dilated cardiomyopathy, thought to have been caused by secondary carnitine deficiency, at the age of two years [7]. Some groups have suggested the use of carnitine supplementation and dietary protein restriction for confirmed cases, but the lack of significant symptoms developing in prospectively followed patients diagnosed from newborn screening programmes, as well as the dearth of clinically presenting cases reported in the literature, has meant that these practices are not widely followed, most centres advocating careful follow-up only [8]. To our knowledge no patient with IBDD has previously been reported to have developed hypoglycaemia. Here we present a case of IBDD diagnosed in the United Kingdom, where C4-carnitine is not part of the newborn screening programme, who presented with ketotic hypoglycaemic encephalopathy during an intercurrent gastroenteritis illness.

## 2. Methods

### 2.1. Ethics

This article does not contain any experimental studies with human or animal subjects performed by any of the authors and therefore ethical

* Corresponding author at: Birmingham Children's Hospital, Steelhouse Lane, Birmingham B4 6NH, United Kingdom.
*E-mail address:* Saikat.santra@bch.nhs.uk (S. Santra).

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approval was not required for this report. Informed consent for publication was obtained from the family of the child included in the report.

### 2.2. ACAD8/ACADS sequencing

PCR amplification of all *ACAD8* exons and part of the flanking introns was performed as described elsewhere [9]. PCR fragments were sequenced in both directions on a 3100-Avant genetic analyzer using BigDye® Terminator v1.1 Cycle Sequencing kit, and using NM_014384.2 as a reference sequence

Rare variants were identified based on a comparison against allelic frequencies from dbSNPv142, and Exome Aggregation Consortium (ExAC).

## 3. Results

### 3.1. Case report

The patient was a 13-month-old female who was born at term by normal spontaneous vaginal delivery to a mother of Pakistani origin. The patient's maternal great-grandmother and paternal grandfather were first cousins. There had been no clinical or developmental concerns with the child over the first year of life. 48 h prior to admission she had received vaccination against measles, mumps and rubella and developed low-grade post-immunisation pyrexia. The next day she developed non-bilious vomiting and diarrhoea which continued for more than 24 h. At the same time, her appetite was poor and she drank water only. During the four hours prior to admission, the child was drowsy and difficult to rouse and she was brought to the hospital emergency department where initial investigations revealed a moderate metabolic acidosis (pH 7.29, base excess −8.5 mmol/l) with hypoglycaemic encephalopathy (blood glucose 1.9 mmol/l). She had rapid clinical improvement with intravenous dextrose administration and faecal analysis subsequently confirmed that rotavirus caused gastroenteritis. No further hypoglycaemia developed after reintroduction of oral feeding and she was discharged home three days later.

Metabolic investigations collected at the time of hypoglycaemia demonstrated an appropriate ketotic response (free fatty acids 2594 μmol/l, 3-hydroxybutyrate 3415 μmol/l) and mildly elevated lactate (3.4 mmol/l [reference range 0.6–2.6]). Blood spot and plasma acylcarnitine analysis revealed an unusually prominent peak of C4-carnitine as well as increases in a number of acylcarnitines secondary to ketosis. Free carnitine was normal (25 μmol/l [reference range 15–53]) Organic acids during illness demonstrated features of ketosis with grossly elevated 3-hydroxybutyrate and acetoacetate as well as a dicarboxylic aciduria.

After recovery, C4-carnitine remained increased and isobutyrylglycine was detected on urine organic acid analysis. Fatty acid oxidation flux studies demonstrated normal oxidation of myristate and oleate in cultured fibroblasts though there was evidence for a partial defect in short chain fatty acid oxidation (butyrate release 0.93 pmol $CO_2$/min/mg protein, normal controls 2.4–19.4).

### 3.2. ACAD8 and ACADS sequencing

DNA sequencing of *ACAD8* identified a novel homozygous missense c.845C > T substitution. This is predicted to cause a single amino acid substitution of serine at position 282 to phenylalanine (p.Ser282Phe). The serine-282 residue is highly conserved across vertebrate species supporting the pathogenicity of this variant. This variant is reported only 6 times in ExAC and only in individuals of South Asian origin with no homozygotes reported, giving an allele frequency of 0.0003634 in this ethnicity. It is listed as rs770663870 in dbSNP (http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=rs770663870). The variant is predicted to be deleterious *in silico* by both Polyphen and SIFT. Parental consent to DNA testing was not provided to confirm homozygosity. DNA sequencing of *ACADS* did not reveal any mutations.

### 3.3. Clinical course

The child was provided with a 15% glucose polymer emergency regimen once the diagnosis was suspected and this has been increased appropriately with age. No change was made to the child's normal daily diet.

The child has been followed up regularly for the ten years since diagnosis and has remained well. There have been no further episodes of hypoglycaemia and in fact the emergency regimen has never actually been required. The child's free carnitine has measured between 25 and 31 μmol/l on follow-up and carnitine supplementation has not been deemed necessary. Serial full blood count estimations have shown no sign of anaemia (Haemoglobin 127 g/l [reference range 120–160] at 11 years of age). Serial echocardiography has shown no evidence of cardiomyopathy over the ten years of follow-up with a shortening fraction in M-mode of 32% at 11 years of age. There have been no concerns about the child's development and she has remained in mainstream education throughout.

## 4. Discussion

Persistent elevations of C4-carnitine may suggest disturbance in short chain fatty acid oxidation (*e.g.* SCADD) leading to butyrylcarnitine accumulation or a disturbance in valine metabolism (*e.g.* IBDD) leading to isobutyrylcarnitine accumulation. The overwhelming majority of patients with IBDD detected from newborn screening programmes have been clinically asymptomatic and it remains uncertain how significant SCADD is as a cause of hypoglycaemia in childhood, though disturbance in longer chain fatty acid oxidation is certainly associated with hypoglycaemia. This child presented with ketotic hypoglycaemic encephalopathy during an episode of gastroenteritis and metabolic abnormalities present at the time of hypoglycaemia were investigated further leading to a diagnosis of IBDD. It remains uncertain whether IBDD contributed to hypoglycaemia in this patient. Disturbed valine catabolism would not be expected to be associated with hypoglycaemia. IBD is, however, one member of the acyl-CoA dehydrogenase family and there is significant sequence and structural similarity across members of this family [9]. Indeed it is recognised that some acyl-CoA dehydrogenases are able to catalyse the metabolism of substrates for other members of the family, with reduced affinity [1,10]. For example medium chain acyl-CoA dehydrogenase is known to have detectable but low activity towards longer chain acyl-CoA molecules. IBD and SBCAD are also known to have some cross-reactivity with their respective substrates [1, 11]. It is therefore tempting to suggest that IBD and SCAD may have some cross-reactivity but cross-reactivity between an enzyme involved in amino acid catabolism and an enzyme involved in fatty acid oxidation has not been described. The structure of IBD has been elegantly elucidated and whilst the structure shares many similarities to that of SCAD, the substrate binding site is wider to allow stable binding of molecules with a 2-methyl group rather than the narrower SCAD substrate binding site which favours straight-chain molecules [11]. Purified recombinant *ACAD8* enzyme has been shown to be virtually inactive towards butyryl-CoA *in vitro* [9] however fibroblasts from this patient did demonstrate a partial defect in butyrate oxidation and the reason for this is uncertain.

Hypoglycaemia in IBDD has not been reported in other patients diagnosed from newborn screening programmes or otherwise, nor was it seen as a recurrent phenomenon in this child. Even if there were some ability of IBD to metabolise short chain fatty acids *in vivo* one would expect the normal activity of SCAD to compensate for deficiency of this seen in a patient with IBDD. With this in mind it is relevant to note that the first reported case of IBDD, and many other cases detected through newborn screening, have additionally been at least

heterozygous for the common c.625G > A variation in *ACADS* [4] which could conceivable have a bearing on the likelihood of clinical problems developing. This was not, however, the case in this child in whom *ACADS* sequencing revealed no pathogenic variant. Furthermore hypoglycaemia is known to complicate the clinical course of infants with severe rotavirus gastroenteritis even without any underlying inborn error of metabolism [12]. It is more likely, therefore, that this child's hypoglycaemia was not primarily caused by IBDD although a contributing role of this condition in early life cannot be excluded. Some recently discovered inborn errors of metabolism have been shown to be associated with clinical symptoms only during infancy [13] with cases remaining asymptomatic in later life and it is conceivable that such mechanisms may also affect the expression of clinical symptoms in IBDD. Furthermore, the normal developmental, haematological and cardiac follow-up of this child suggests that previously reported learning difficulties, anaemia and cardiomyopathy are not necessarily proven associations with IBDD, though this child did not have systemic carnitine deficiency either at presentation or on follow-up.

## 5. Conclusions

In conclusion, the clinical significance of IBDD is uncertain and it remains a dilemma for clinicians managing children diagnosed through newborn screening programmes. Very few cases presenting with clinical symptoms are reported in the literature. We present the case of a child who was diagnosed through investigation for ketotic hypoglycaemia. In retrospect whilst it is unlikely that IBDD solely caused the hypoglycaemia in this patient, it is possible that this episode was more pronounced due to IBDD. Given the uncertainty of the natural history of IBDD, however, we would recommend patients are followed-up carefully and glucose polymer emergency regimens may be indicated if recurrent episodes of hypoglycaemia occur.

## Conflict of interest

Dr. Saikat Santra, Mrs. Mary Anne Preece, Dr. Rikke K. Olsen and Prof. Brage S. Andresen declare that they have no conflict of interest. Professor Anita Macdonald has received research grants from Vitaflo and Nutricia and is on an advisory group for Nutricia.

## Informed consent and animal rights

This article does not contain any experimental studies with human or animal subjects performed by any of the authors and therefore ethical approval was not required for this report. Informed consent for publication was obtained from the family of the child included in the report.

## Details of the contributions of individual authors

All authors, have contributed to the planning, conduct, and reporting of the work described in this article. Dr. Saikat Santra as principal author will serve as guarantor for the article and accepts full responsibility for the work. Dr. Santra wrote the manuscript, has access to the data, and controlled the decision to publish. Professor Anita Macdonald reviewed the manuscript and contributed details of dietary management. Mrs. Mary Anne Preece reviewed the manuscript and contributed details of laboratory investigations. Dr. Rikke K. Olsen and Professor Brage S Andresen reviewed the manuscript and contributed details of molecular investigations. The authors confirm independence from any sponsor; the content of the article has not been influenced by any sponsor.

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