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CLINICAL REPORT

Isobutyryl-CoA dehydrogenase deficiency associated with autism in a girl without an alternative genetic diagnosis by trio whole exome sequencing: A case report

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Abstract

Background: Isobutyryl-CoA dehydrogenase (IBD) is a mitochondrial enzyme catalysing the third step in the degradation of the essential branched-chain amino acid valine and is encoded by *ACAD8*. *ACAD8* mutations lead to isobutyryl-CoA dehydrogenase deficiency (IBDD), which is identified by increased C4-acylcarnitine levels. Affected individuals are either asymptomatic or display a variety of symptoms during infancy, including speech delay, cognitive impairment, failure to thrive, hypotonia, and emesis.

Methods: Here, we review all previously published IBDD patients and describe a girl diagnosed with IBDD who was presenting with autism as the main disease feature.

Results: To assess whether a phenotype-genotype correlation exists that could explain the development or absence of clinical symptoms in IBDD, we compared CADD scores, in silico mutation predictions, LoF tolerance scores and C4-acylcarnitine levels between symptomatic and asymptomatic individuals. Statistical analysis of these parameters did not establish significant differences amongst both groups.

Conclusion: As in our proband, trio whole exome sequencing did not establish an alternative secondary genetic diagnosis for autism, and reported long-term follow-up of IBDD patients is limited, it is possible that autism spectrum disorders could be one of the disease-associated features. Further long-term follow-up is suggested in order to delineate the full clinical spectrum associated with IBDD.

KEYWORDS

autism, genotype-phenotype correlation, isobutyryl-CoA dehydrogenase deficiency, whole exome sequencing

1 | INTRODUCTION

Isobutyryl-CoA dehydrogenase (IBD), encoded by the *ACAD8* gene (OMIM #604773) on chromosome 11q25, belongs to the Acyl-CoA dehydrogenase (ACADs) family which is a group of mitochondrial enzymes involved in the catabolism of fatty acids and branched-chain amino acids (Ikeda et al., 1983). It is responsible for the conversion of isobutyryl-CoA to methylacrylyl-CoA at the third step in the catabolism of the essential branched-chain amino acid valine (Andresen et al., 2000). Isobutyryl-CoA dehydrogenase deficiency (OMIM #611283, IBDD) (Roe et al., 1998) is a rare autosomal recessive disorder that is caused by bi-allelic mutations in *ACAD8*, which reduce or eliminate the ability of IBD to catabolize valine (Andresen et al., 2000). IBDD causes blockage of valine oxidation resulting in the accumulation of isobutyryryl-CoA, followed by transesterification with carnitine which leads to the formation of C4-acylcarnitine and free CoA and excretion of acylcarnitines in urine (Reuter & Evans, 2012). In some cases, carnitine re-uptake by the carnitine transporter in renal cells is inhibited, resulting in systemic secondary depletion of carnitine (Reuter & Evans, 2012). Therefore, IBDD patients present with accumulation of C4-acylcarnitine in plasma and urine and in some cases secondary carnitine deficiency.

IBDD, in most cases, is suspected after initial aberrant newborn screening (NBS) performed by tandem mass spectrometry (MS/MS) to determine C4-acylcarnitine levels which may represent isobutyrylcarnitine or butyrylcarnitine. However, elevated levels of C4-acylcarnitine are not IBDD specific and are also observed in short-chain acyl-CoA dehydrogenase deficiency and ethylmalonic encephalopathy (Zafeiriou et al., 2007). In vitro probe studies of fibroblast fatty acid oxidation and specific detection of isobutyrylglycine in urine can help to distinguish between these disorders. However, final diagnosis of IBDD requires isobutyryl-CoA dehydrogenase activity determination or genetic testing for mutations in *ACAD8* (Koeberl et al., 2003). Affected individuals are reported to be either asymptomatic or develop symptoms during infancy or childhood, such as mild intellectual disability, speech delay, and failure to thrive with emesis (Koeberl et al., 2003; Lin et al., 2018; Oglesbee et al., 2007; Pedersen et al., 2006; Roe et al., 1998; Santra et al., 2017; Sass et al., 2004). Since, most cases of IBDD reported in literature have been identified through expanded NBS and limited data on their clinical follow-up is available, at present the complete clinical spectrum of this disorder is undefined. Here, we review all previously described IBDD cases and report a girl presenting with autism, diagnosed with IBDD upon metabolic and targeted genetic investigation, in which subsequent trio whole exome sequencing (WES) did not establish an alternative genetic diagnosis that could explain autism.

2 | METHODS

2.1 | Ethical compliance

Parents gave written informed consent for publication of anonymized medical data and clinical photographs of the proband, collected in a clinical care setting. All metabolic investigations were performed in a clinical diagnostic setting. Use of genome-wide genetic investigations, including trio WES in a clinical setting, was approved by the Erasmus MC Institutional Review Board (METC-2012-387).

2.2 | Trio whole exome sequencing

Trio WES was performed and analysed as previously described (Hengel et al., 2020; Perenthaler et al., 2020). In short, genomic DNA was isolated from peripheral blood leukocytes of the proband and both parents and exome-coding DNA was captured with the Agilent SureSelect Clinical Research Exome (CRE) kit (v2). Sequencing was performed on an Illumina HiSeq 4000 with 150 bp paired end reads. Reads were aligned to hg19 using BWA (BWA-MEM v0.7.13) and variants were called using the GATK haplotype caller (v3.7 (reference: http://www.broadinstitute.org/gatk/). Detected variants were annotated, filtered and prioritized using the Bench lab NGS v5.0.2 platform (Agilent technologies). Initially, only genes known to be involved in intellectual disability were analyzed, followed by a full exome analysis. The encountered *ACAD8* variant (reference transcript NM_014384.2) was verified by Sanger sequencing using the following sequencing primers: ACAD8_03_F (TGTAAAACGACGG CCAGTCCTCACTGTGCCCTCTAAA), ACAD8_03_R (CAGGAAACAGCTATGACCTACGAATCTGAA CTCTCACAGTC).

2.3 | Biochemical analysis

Acylcarnitine concentrations in plasma and urine were measured by flow-injection tandem mass spectrometry (Vreken et al., 1999). Routine screening of urine organic acids was performed by gas chromatography-mass spectrometry of methyl derivatives.

2.4 | Literature search

Literature on IBDD was searched in PubMed (last assessed: 13 June 2020), focusing on publications in English. This resulted in 41 publications, of which 17 were dealing with patients affected with IBDD and were, therefore, included in our review.

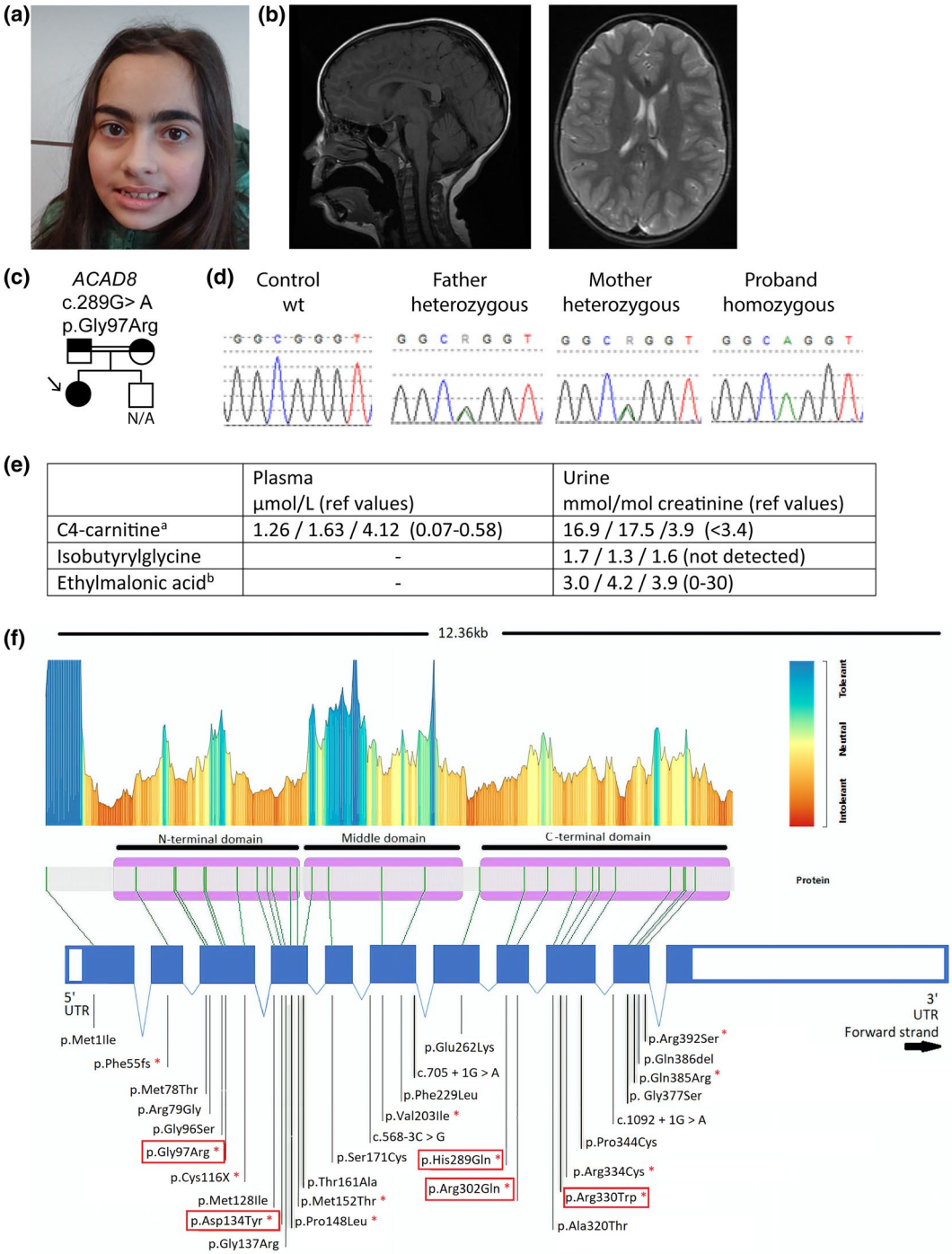
2.5 | In silico analysis and genotype-phenotype correlation

Combined Annotation Dependent Depletion (CADD) scores (v1.4) (Kircher et al., 2014), representing the deleteriousness of single nucleotide variants and insertion/deletions variants in the human genome, were retrieved for each variant found in IBDD patients from https://cadd.gs.washington.edu/. MutationTaster (Schwarz et al., 2014) was used with default settings (http://www.mutationtaster.org/). To determine LoF tolerance and display encountered variants in *ACAD8*, MetaDome (Wiel et al., 2019) (https://stuart.radboudumc.nl/metadome/), was used, as we described before (Nabais Sá et al., 2020). To determine whether mutation characteristics were different between asymptomatic and symptomatic individuals, the average CADD and LoF score for both groups was calculated (summing up values from both alleles per individual) and the 95% confidence interval was calculated to assess whether differences were significant ($p < .05$). To assess a possible correlation between C4-acylcarnitine levels detected by MS/MS blood spot in NBS and the development of clinical symptoms in IBDD patients, the average C4-acylcarnitine levels were compared between symptomatic versus asymptomatic group and the differences assessed using the same statistics.

3 | RESULTS

3.1 | Case report

The proband is a currently 11-year-old girl (Figure 1a), born by vacuum extraction at 40 weeks of gestation as the first child to distantly related Turkish, healthy parents. Pregnancy was uneventful, and birth weight was 3585 gram (p50). The start was normal, and no congenital anomalies or major dysmorphic features were noticed. A 4-year-old younger brother is healthy and has no medical issues, with no other cases of autism known in the family. The first year of the girl was uneventful. Motor development was normal, with independent ambulation at the age of 12 months. Parents noticed lack of interaction and lack of social eye contact early on. At the age of 2 years and 5 months, she first came to medical attention due a severe lack of speech development, which was assumed to be caused by hearing problems. At that age, she only expressed a few, barely understandable words and made some sounds. However, Extensive ENT investigations were normal, after which, at the age of 2 years and 7 months, a multidisciplinary neuropsychological assessment was performed showing internalizing behavior and a lack of social interactions. Further child psychiatry assessment lead to the diagnosis of autism at the age of 3 years (DSM-IV classification: axis I:299.00; axis II: 799.90, axis III: no somatic disorder; axis IV: bilingual education; axis V:cGas:35). Pivotal Response Treatment led to some improvements in social communication, allowing her to follow pre-school medical day care and improving in play interactions with other children. Toilet training was achieved at the age of 5 years. At the age of 5 years and 4 months, she was referred to the neurology department for assessment of a cause of her autism. At that age, she was described as a quiet child, being in her own world, and speaking few words. Motor development was normal, and no focal neurological abnormalities were seen. An EEG was normal. A brain MRI at the age of 5 years and 10 months showed a structurally normal brain (Figure 1b), with no signs of aberrant neuronal migration or metabolic disorders, and no signs of previous asphyxia. Routine blood investigations and FGF-21 in serum were normal. SNP-array analysis revealed several runs of homozygosity (ROH, in total 42 Mb) in line with the distant consanguinity between parents, and a not-previously reported variant of unknown significance (loss of approximately 533 kb in band 7p15.3, arr 7p15.3(22,126,627-22,659,465) x1 (hg18)), which was inherited from the unaffected father. Metabolic testing showed increased C4-carnitine in plasma and urine, increased isobutyrylglycine and decreased C4-carnitine/isobutyryl-carnitine ratios, all suggestive of IBDD (Figure 1e). Subsequent next generation sequencing based gene panel analysis of genes implicated in metabolic diseases found a homozygous variant in *ACAD8* (NM_014384.2 (ACAD8): c.289G> A, p.Gly97Arg) (Figure 1c,d). This variant is found nine times heterozygous but not homozygous in GnomAD (MAF 0.0000358), is predicted to be disease causing by MutationTaster (Schwarz et al., 2010), has a CADD score (v1.4) of 31 (Kircher et al., 2014) and has previously been identified in three affected individuals with IBDD (Oglesbee et al., 2007; Santra et al., 2017; Yun et al., 2015), thereby confirming the diagnosis of IBDD in our proband. Subsequent suppletion with carnitine (2x daily, 500 mg) lead to a subjective increase in appetite but did not improve the autism phenotype. Cardiologic evaluation, including ECG and cardiac ultrasound did not show any abnormalities. Investigation at the Clinical Genetics outpatient clinic at the age of 8 years and 7 months showed stable normal growth (with head circumference, height and weight all between 0 and −1 SD at multiple measurements over the years), and no major dysmorphic features other than a mild 2–3 toe syndactyly. She was following special education, and speech was limited to a few words and noises. Given the severity of autism, a possible second genetic disorder was considered. Therefore, trio WES was performed in a clinical setting, which passed all clinical grade quality controls for sequencing coverage. Analysis first focused on a panel of ~1,200 genes involved in intellectual disability, followed by a complete open exome analysis. A variant of unknown



	Plasma µmol/L (ref values)	Urine mmol/mol creatinine (ref values)
C4-carnitine[a]	1.26 / 1.63 / 4.12 (0.07-0.58)	16.9 / 17.5 /3.9 (<3.4)
Isobutyrylglycine	-	1.7 / 1.3 / 1.6 (not detected)
Ethylmalonic acid[b]	-	3.0 / 4.2 / 3.9 (0-30)

FIGURE 1 (a) Facial image of IBDD proband. (b) Midsagital T1 and axial T2 weighted brain MRI of the IBDD proband, showing normal structural brain morphology. (c) Family pedigree showing segregation of the *ACAD8* variant; N/A, not available for genetic testing. (d) Chromatogram showing the *ACAD8* c.289G> A, p.Gly97Arg variant (NM_014384.2) in a homozygous state in the proband and in a heterozygous state in both parents. (e) Overview of metabolic investigations. [a]Butyryl-carnitine + isobutyryl-carnitine [b]Ethylmalonic acid is normal in IBDD, but elevated in SCAD or ETHE1 deficiency. (f) Mutational spectrum of *ACAD8* from all described IBDD patients. *ACAD8* consists of 11 coding exons (blue). Variants identified in symptomatic patients are marked (*), red boxed variants are found in a homozygous state in symptomatic individuals. LoF tolerance landscape from MetaDome analysis is indicated

significance in *DNA2* (OMIM 601810, NM_001080449.2 (DNA2) c.2036-2037 ins AA, p. (His679Glnfs*10)) inherited from the unaffected mother was found, but besides the previously identified homozygous *ACAD8* variant no other likely disease implicated variant was identified. Both parents were heterozygous carriers of the *ACAD8* variant. The unaffected brother was not available for genetic investigations.

TABLE 1 Overview of described IBDD patients

Patient no.	Sex	Zygosity	Genomic variant		Protein variant		CADD score		LoF tolerance score		Clinical state at birth	Clinical symptoms	Metabolic findings								
			Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2			C4-acylcarnitine in blood spot MS/MS(NBS)	NBS results (μmol/L)	Day of NBS	Plasma C4 acylcarnitine profile	Urine Isobutyryl-glycine	Urine C4-acylcarnitine	Isobutyrylcarnitine (Fibroblasts FAO)	Last follow-up age (years)	References
1	F	hom	c.905G>A	c.905G>A	Arg302Gln	Arg302Gln	32	32	0.96	0.96	Unremarkable	Failure to thrive, congenital heart malformation, dilated cardiomyopathy, anemia	ND (later identified)	ND (later identified)	ND	↑ (after L-carnitine supplement)	↑ (after L-carnitine supplement)	ND	↑	11	Nguyen et al. (2002), Pedersen et al. (2006), Roe et al. (1998)
2	F	het	c.163_164insCT	c.607G>A	Phe55fs,	Val203Ile	ND	23.4	0.5	0.74	Unremarkable	Developmental delay/ intellectual disability, hypotonia	↑	0.95/0.58	2/14	↑	↑	↑	ND	0.7	Pedersen et al. (2006), Sass et al. (2004)
3	M	hom	c.384G>C	c.384G>C	Met128Ile	Met128Ile	29	29	0.36	0.36	Unremarkable	Normal	↑	0.92/1.55	4/12	↑	↑	↑	ND	1.1	Pedersen et al. (2006), Sass et al. (2004)
4	ND	ND	c.443C>T	ND	Pro148Leu	ND	20.3	ND	0.5	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Battaile et al. (2004), Pedersen et al. (2006)
5	ND	ND	c.988C>T	ND	Arg330Trp	ND	23.1	ND	0.68	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	Battaile et al. (2004), Pedersen et al. (2006)
6	F	het	c.409G>A	c.958G>A	Gly137Arg	Ala320Thr	32	29.7	0.41	0.58	Unremarkable	Normal	↑†	1.1/0.8	8/24	↓†	↑	ND	ND	2.5	Pedersen et al. (2006)
7	F	het	c.455T>C	c.1154A>G	Met152Thr	Gln385Arg	32	29	0.45	0.75	Unremarkable	Hypotonia, congenital heart malformation	↑†	2.9/2.6	1/8	↑	Normal	ND	↑	3.8	Pedersen et al. (2006)
8	F	het	c.348C>A	c.1000C>T	Cys116X	Arg334Cys	35	29.8	0.59	0.5	Unremarkable	Speech delay	↑†	3.23/2.33	2/8	↑†	↑	ND	↑	At least 5	Koeberl et al. (2003), Pedersen et al. (2006)
9	M	hom	c.400G>T	c.400G>T	Asp134Tyr	Asp134Tyr	33	33	0.39	0.39	Unremarkable	Speech delay, lethargy, ear infections	↑†	2.41/2.40	5/37	↑†	↑	ND	ND	At least 2	Pedersen et al. (2006)
10	M	het	c.3G>T	c.1000C>T	Met1Ile	Arg334Cys	18.17	29.8	1.41	0.5	Unremarkable	Normal	↑	3.89	18	↑†	ND	↑†	ND	2.9	Yoo et al. (2007)
11	F	hom	c.988C>T	c.988C>T	Arg330Trp	Arg330Trp	23.1	23.1	0.68	0.68	Unremarkable	Emesis, gastroenteritis, ear infections	↑	2.9	ND	↑	Normal	ND	↑	5	Oglesbee et al. (2007)
12	F	het	c.289G>A	c.455T>C	Gly97Arg	Met152Thr	31	32	0.76	0.45	Unremarkable	unremarkable	↑	2.5	ND	↑	ND	ND	↑	5	Oglesbee et al. (2007)
13	M	hom	c.867C>A	c.867C>A	His289Gln	His289Gln	11.88	11.88	0.65	0.65	Unremarkable	Neonatal hyperbilirubinemia	↑	2.4	4	↑	Normal	ND	↑	6.5	Oglesbee et al. (2007), Pena et al. (2012)
14	F	hom	c.867C>A	c.867C>A	His289Gln	His289Gln	11.88	11.88	0.65	0.65	Unremarkable	Normal	↑	2.1	9	↑	Normal	↑	ND	4.6	Oglesbee et al. (2007), Pena et al. (2012)
15	F	het	c.443C>T	c.455T>C	Pro148Leu	Met152Thr	20.3	32	0.5	0.45	Unremarkable	Emesis, pyelonephritis, gastroenteritis	↑	2	17	↑	ND	ND	↑	6.3	Oglesbee et al. (2007), Pena et al. (2012)
16	F	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	↑	2	ND	↑	Normal	↑	ND	5	Oglesbee et al. (2007)
17	F	het	c.958G>A	c.1129G>A	Ala320Thr	Gly377Ser	29.7	33	0.58	0.6	ND	ND	↑	2	ND	↑	Normal	↑	↑	5	Oglesbee et al. (2007)
18	M	het	c.687T>G	c.1129G>A	Phe229Leu	Gly377Ser	22.8	33	0.97	0.6	Unremarkable	Normal	↑	2.2	7	↑	Normal	↑	↑	3.3	Oglesbee et al. (2007), Pena et al. (2012)

(Continues)

TABLE 1 (Continued)

Patient no.	Sex	Zygosity	Genomic variant		Protein variant		CADD score		LoF tolerance score		Clinical state at birth	Clinical symptoms	Metabolic findings								
			Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2	Allele 1	Allele 2			C4-acylcarnitine in blood spot MS/MS(NBS)	NBS results (μmol/L)	Day of NBS	Plasma C4 acylcarnitine profile	Urine Isobutyryl-glycine	Urine C4-acylcarnitine	Isobutyrylcarnitine (Fibroblasts FAO)	Last follow-up age (years)	References
19	M	ND	ND	ND	ND	ND	ND	ND	ND	ND	Unremarkable	Asthma	ND	ND (later identified)	ND	↑	ND	ND	ND	6.8	Oglesbee et al. (2007), Pena et al. (2012)
20	M	het	c.455T>C	c.512C>G	Met152Thr	Ser171Cys	32	25.3	0.45	1.08	Unremarkable	Normal	↑	1.8	ND	↑	Normal	↑	↑	5	Oglesbee et al. (2007)
21	M	hom	c.233T>C	c.233T>C	Met78Thr	Met78Thr	25.9	25.9	0.61	0.61	Unremarkable	Normal	↑	1.8	ND	↑	Normal	↑	↑	5	Oglesbee et al. (2007)
22	F	ND	ND	ND	ND	ND	ND	ND	ND	ND	Unremarkable	Normal	↑	2.7	7	↑	↑	↑	ND	0.7	Oglesbee et al. (2007), Pena et al. (2012)
23	M	ND	ND	ND	ND	ND	ND	ND	ND	ND	Unremarkable	Normal	↑	1.9	8	↑	Normal	↑	ND	1.8	Oglesbee et al. (2007), Pena et al. (2012)
24	ND	hom	c.1129G>A	c.1129G>A	Gly377Ser	Gly377Ser	33	33	0.6	0.6	Unremarkable	Normal	↑	2.26	1–3	↑	↑	ND	ND	ND	Scolamiero et al. (2015)
25	ND	het	c.289G>A	c.1156_1158delCAG	Gly97Arg	Gln386del	32	ND	0.76	0.79	Unremarkable	Normal	↑	1.67	ND	ND	↑	ND	ND	ND	Yun et al. (2015)
26	ND	het	c.3G>T	c.1156_1158delCAG	Met1Ile	Gln386del	18.17	ND	1.41	0.79	Unremarkable	Normal	↑	2.57	ND	ND	↑	ND	ND	ND	Yun et al. (2015)
27	F	hom	c.289G>A	c.289G>A	Gly97Arg	Gly97Arg	31	31	0.76	0.76	Unremarkable	Emesis, failure to thrive, hypoglycemic encephalopathy, gastroenteritis	††	ND	ND	††	Normal	ND	Normal	11	Santra et al. (2017)
28	F	het	c.235C > G	c.1000C > T	Arg79Gly	Arg334Cys	25.4	29.8	0.5	0.5	Unremarkable	Normal	↑	1.47/1.31	4/13	↑	ND	ND	ND	1.6	Lin et al. (2018, 2019)
29	M	hom	c.286G > A	c.286G > A	Gly96Ser	Gly96Ser	29.7	29.7	0.6	0.6	Unremarkable	Normal	↑	1.94/1.69	4/11	↑	ND	ND	ND	1.6	Lin et al. (2018, 2019)
30	F	hom	c.286G > A	c.286G > A	Gly96Ser	Gly96Ser	29.7	29.7	0.6	0.6	Unremarkable	Normal	↑	1.29/1.96	7/21	↑	ND	ND	ND	1.4	Lin et al. (2018, 2019)
31	M	het	c.286G > A	c.444G > T	Gly96Ser	Pro148Pro	29.7	10.7	0.6	0.5‡	Unremarkable	Normal	↑	0.98/0.77	4/21	↑	ND	ND	ND	1.2	Lin et al. (2018, 2019)
32	F	het	c.286G > A	c.1092 + 1G > A	Gly96Ser	Splice site mutation	29.7	32	0.6	ND	Unremarkable	Normal	↑	0.83/1.38	4/20	↑	Normal	ND	ND	0.8	Lin et al. (2018, 2019)
33	M	het	c.286G > A	c.1092 + 1G > A	Gly96Ser	Splice site mutation	29.7	32	0.6	ND	Unremarkable	Speech delay, learning disability	↑	ND (later identified)	ND	↑	ND	ND	ND	8.9	Lin et al. (2018, 2019)
34	M	het	c.444G > T	c.1176G > T	Pro148Pro	Arg392Ser	10.7	25.4	0.5‡	0.53	Unremarkable	Hypotonia, emesis, hematemesis, failure to thrive	↑	1.01/0.98	10/19	↑	ND	ND	ND	0.5	Lin et al. (2018, 2019)
35	ND	het	c.286C > A	c.1000C > T	Pro344Cys	Gly96Ser	29.7	29.8	0.58	0.6	Unremarkable	Normal	↑	ND	ND	ND	ND	ND	ND	ND	T. Wang et al. (2019)
36	ND	het	c.286C > A	c.1000C > T	Pro344Cys	Gly96Ser	29.7	29.8	0.58	0.6	Unremarkable	Normal	↑	ND	ND	ND	ND	ND	ND	ND	T. Wang et al. (2019)
37	ND	het	c.568-3C > G	c.1000C > T	Frameshift	Pro344Cys	15.29	29.8	0.58	ND	Unremarkable	Normal	↑	ND	ND	ND	ND	ND	ND	ND	T. Wang et al. (2019)
38	ND	het	c.705 + 1G > A	c.1176G > T	Frameshift	Arg392Ser	ND	25.4	1.91	0,53	Unremarkable	ND	↑	ND	ND	ND	ND	ND	ND	ND	W. Wang et al. (2019)
39	ND§	hom	c.384G > A	c.384G > A	Met128Ile	Met128Ile	28.3	28.3	0.36	0.36	Unremarkable	Normal	↑	1.8	ND	↑	ND	ND	ND	ND¶	Sadat et al. (2020)
40	ND§	hom	c.481A > G	c.481G > A	Thr161Ala	Thr161Ala	5.217	5.217	1.22	1.23	Unremarkable	Normal	↑	1.5	ND	↑	ND	ND	ND	ND¶	Sadat et al. (2020)
41	ND§	het	c.400G > T	c.784G > A	Asp134Tyr	Glu262Lys	33	22.8	0.36	0.42	Unremarkable	Normal	↑	3.5	ND	↑	ND	ND	ND	ND¶	Sadat et al. (2020)
42	ND§	het	c.400G > T	c.784G > A	Asp134Tyr	Glu262Lys	33	22.8	0.36	0.43	Unremarkable	Normal	↑	2.5	ND	↑	ND	ND	ND	ND¶	Sadat et al. (2020)
43	ND§	hom	c.905G > A	c.905G > A	Arg302Gln	Arg302Gln	32	32	0.96	0.97	Unremarkable	Normal	↑	1.4	ND	↑	ND	ND	ND	ND¶	Sadat et al. (2020)
44	ND§	ND	ND	ND	ND	ND	ND	ND	ND	ND	Unremarkable	Normal	↑	1.7	ND	↑	ND	ND	ND	ND¶	Sadat et al. (2020)

(Continues)

TABLE 1 (Continued)

Patient no.	Sex	Genomic variant		Protein variant		CADD score		LoF tolerance score		Metabolic findings								Last follow-up age (years)	References
		Zygosity	Allele 1	Allele 2	Allele 1	Allele 1	Allele 2	Allele 1	Allele 2	Clinical state at birth	Clinical symptoms	C4-acylcarnitine in blood spot MS/MS(NBS)	NBS results (μmol/L)	Day of NBS	Plasma C4 acylcarnitine profile	Urine Isobutyryl-glycine	Urine C4-acylcarnitine	Isobutyrylcarnitine (Fibroblasts FAO)	
45	ND§	ND	ND	ND	ND	ND	ND	ND	ND	Unremarkable	Normal	↑	1.7	ND	↑	ND	ND	ND	ND¶	Sadat et al. (2020)
46	ND§	ND	ND	ND	ND	ND	ND	ND	ND	Unremarkable	Normal	↑	1.6	ND	↑	ND	ND	ND	ND¶	Sadat et al. (2020)
47	F	hom	c.289G>A	c.289G>A	Gly97Arg	Gly97Arg	32	32	0.76	0.76	Unremarkable	Developmental delay/ intellectual disability, speech delay, learning disability, autism	ND (later identified)	ND (later identified)	ND	↑†	↑	↑†	ND	11	This report

Notes: IBDD patients described in literature including sex, zygosity, genomic and protein variants, CADD scores and LoF tolerance score for each variant. Clinical state at birth and symptoms reported later in life are displayed. Previously reported metabolic findings for each case are displayed, including blood spot MS/MS analysis, plasma acylcarnitine profile, metabolic findings in urine and Fibroblasts fatty acid oxidation (FAO) probe studies. The reported age at last follow-up age of each individual is also presented. ND, no data; hom, homozygous; het, compound heterozygous; later identified, patients not identified by NBS; FAO, fatty acid oxidation; ↑, increased; † C4-carnitine. ‡ mutation leading to a synonymous aminoacid change. § The sex of each patient was not described, but out of the eight patients reported in the study of Sadat et al. (2020), four were male and foue were female. ¶ Only a range of the follow-up age of the individuals was provided by Sadat et al. (2020) (1–8 years old).

3.2 | Review of the literature

Including our patient, to date, 47 individuals with IBDD, with a broad variety of ethnic backgrounds, have been described (Battaile et al., 2004; Koeberl et al., 2003; Lin et al., 2018; Nguyen et al., 2002; Oglesbee et al., 2007; Pedersen et al., 2006; Pena et al., 2012; Roe et al., 1998; Sadat et al., 2020; Santra et al., 2017; Sass et al., 2004; Scolamiero et al., 2015; T. Wang et al., 2019; W. Wang et al., 2019; Yoo et al., 2007; Yun et al., 2015) of which 22 are female, 17 are male and for eight cases gender was not reported (Table 1). Metabolic data have been described for 45 individuals, of which 38 have genetically confirmed bi-allelic variants in *ACAD8*. Of these 38 genetically confirmed individuals, 12 showed clinical symptoms, 24 are reported to be asymptomatic, and for two individual no clinical data have been described.

Clinical symptoms reported include neurodevelopmental delay/intellectual disability (2/36), hypotonia (3/36), speech delay (4/36), learning disability (2/36), emesis (4/36), failure to thrive (3/36), congenital heart malformation (2/36), dilated cardiomyopathy (1/36) and others (8/36) (Table 1). The average age at last follow-up was 4.2 years ($SD = 3.1$ years), and for at least 10 patients, no follow-up after the age of 3 years has been reported.

To assess whether a genotype-phenotype correlation exists, we first mapped all reported pathogenic variants in *ACAD8* (Figure 1f). Variants are widely distributed along the gene, including mutations in the N- and C-terminal alpha-helical domain and the medial beta-strand domain, with no clear differences in spatial localisation between symptomatic and asymptomatic individuals. The average CADD score was 27.2, 95%CI [24.2, 30.2], in the symptomatic group compared to 26.7, 95%CI [24.6, 28.7], in the asymptomatic group which was slightly but not significantly lower. Similarly, we did not find a difference in the average LoF tolerance score between the two groups (0.63, 95%CI [0.56, 0.7] and 0.679, 95%CI [0.59, 0.77] in symptomatic and asymptomatic respectively). We next assessed whether a correlation exists between the levels of C4-acylcarnitine and clinical symptoms. The average C4-acylcarnitine levels detected by MS/MS blood spot analysis was 2.124 μmol/L, 95%CI [1.56, 2.59], in the symptomatic group compared to 1.996, 95%CI [1.74, 2.25] in the asymptomatic group. Therefore, no clear genotype-phenotype or biochemical correlation explains phenotypical differences between IBDD patients.

4 | DISCUSSION

Here, we report an individual diagnosed with IBDD and autism, and review all previously described IBDD cases. Whereas the majority of IBDD cases has been reported to be asymptomatic, several individuals have been described

manifesting clinical phenotypes, including neurodevelopmental and speech delay. No clear genotype-phenotype correlation emerged from our analysis, and no association between C4-acylcarnitine levels in NBS and clinical features was identified. Most IBDD individuals were identified during NBS, and reported clinical information and long-term follow-up is limited. Hence, at present, the clinical spectrum of this disorder remains to be elucidated. Although autism has not yet been specifically reported to be associated with IBDD, at least three and one previously reported individuals displayed speech delay or neurodevelopmental delay, respectively (Koeberl et al., 2003; Lin et al., 2018; Pedersen et al., 2006; Sass et al., 2004), and many other individuals were reported at an early age at which a autism diagnosis might not yet have been possible to establish (Johnson et al., 2007). As in the reported symptomatic cases no further genetic analysis has been performed after the IBDD diagnosis, it remains possible that in a number of cases a secondary genetic diagnosis could explain some of the clinical phenotypes. However, in our case, extensive genetic investigations including SNP-array and trio WES, aiming to identify a confounding secondary genetic cause, did not establish an alternative genetic diagnosis. Although we cannot exclude that with the current clinical technology, an alternative genetic diagnosis was missed, for example due to a genetic variant in non-coding regions that are not assessed during WES (Perenthaler et al., 2019), it seems as likely that there is no secondary genetic cause explaining the presence of autism in this individual. Hence, it is possible that autism spectrum features might be associated with IBDD, similar to the occurrence of autism in many other inborn errors of metabolism including those in related pathways (Novarino et al., 2012; Simons et al., 2017). Future long-term follow-up of IBDD cases will be necessary to further delineate the clinical phenotype of this metabolic disorder.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

TSB conceived the study and supervised the work. EM, KS, YB, and TSB collected clinical data. DH, MS, and GR performed genetic and biochemical investigations. ME and TSB performed the literature review and wrote the paper with input from all authors.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request. Primary patient data (including sequencing and biochemical data) cannot be made available due to restrictions by patient consent.

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