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Methylmalonyl-coA epimerase deficiency: A new case, with an acute metabolic presentation and an intronic splicing mutation in the *MCEE* gene

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ABSTRACT

Methylmalonyl-coA epimerase (MCE) follows propionyl-coA carboxylase and precedes methylmalonyl-coA mutase in the pathway converting propionyl-coA to succinyl-coA. MCE deficiency has previously been described in six patients, one presenting with metabolic acidosis, the others with nonspecific neurological symptoms or asymptomatic. The clinical significance and biochemical characteristics of this rare condition have been incompletely defined. We now describe a patient who presented acutely at 5 years of age with vomiting, dehydration, confusion, severe metabolic acidosis and mild hyperammonemia. At presentation, organic acid profiles were dominated by increased ketones and 3-hydroxypropionate, with moderately elevated methylcitrate and propionylglycine, and acylcarnitine profiles showed marked C3 (propionylcarnitine) elevation with normal C4DC (methylmalonylcarnitine + succinylcarnitine). Propionic acidemia was initially suspected, but it was subsequently noted that methylmalonic acid was mildly but persistently elevated in urine, and clearly elevated in plasma and cerebrospinal fluid. The overall biochemical profile prompted consideration of MCE deficiency. Studies on cultured fibroblasts showed moderately decreased propionate incorporation. Complementation analysis permitted assignment to the *MCEE* group. A heterozygous p.Arg47Ter (p.R47*) mutation in the *MCEE* gene was identified by sequencing of exons, and RNA studies identified a novel intronic splicing mutation, c.379-644A > G, confirming the diagnosis of MCE deficiency. Following the initial severe presentation, development has been normal and the clinical course over the subsequent six years has remained relatively uneventful on an essentially normal diet. This report contributes to the clinical and biochemical characterisation of this rare disorder, while highlighting potential causes of under-diagnosis or of diagnostic confusion.

1. Introduction

Metabolism of propionyl-coA to succinyl-coA involves three successive enzymatic reactions (Fig. 1). Inherited defects in the enzymes catalysing the first and third steps of this pathway, and in the metabolism of their respective cofactors, cause well-characterised, clinically significant diseases [1–3]. In contrast, the physiological significance of the second enzymatic step, catalysed by methylmalonyl-coA epimerase (MCE; also known as methylmalonyl-coA racemase), has remained less clear. There is evidence that this reaction may be partially bypassed *in vivo*, by a shunt pathway proceeding *via* free methylmalonic acid [4–5].

MCE deficiency was first identified in 2006. Four cases, from three families, have been described in case reports. One patient presented with acute metabolic decompensation; an older sibling had developmental delay attributable to co-existing hydrocephalus, but was otherwise asymptomatic [5]. Two unrelated patients had progressive neurological symptoms; however, each was also affected by a second inherited disorder, sepiapterin reductase deficiency, which fully explained the clinical course [6–8]. All four patients presented with methylmalonic aciduria which was persistent but usually mild or

Abbreviations: MCE, methylmalonyl-coA epimerase; *MCEE*, gene encoding methylmalonyl-coA epimerase; MMA, methylmalonic acid or methylmalonate; CSF, cerebrospinal fluid; PCC, propionyl-coA carboxylase; MUT, methylmalonyl-coA mutase; C3, C3, (=propionyl) carnitine; C4DC, C4dicarboxyl (=methylmalonyl + succinyl) carnitine.

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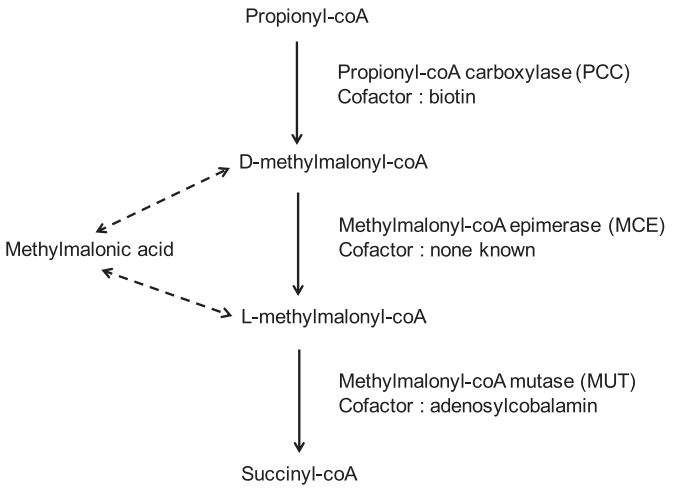

Fig. 1. Metabolism of propionyl-coA to succinyl-coA. The solid arrows depict known direct enzymatic conversions. The hatched arrows represent a proposed alternative shunt pathway. Enzyme Commission numbers are as follows: PCC, EC 6.4.1.3; MCE, EC 5.1.99.1; MUT, EC 5.4.99.2.

moderate. Two additional cases were identified by sequencing of the *MCEE* gene in fibroblasts banked from over 200 patients with methylmalonic aciduria of unknown cause [9]. One of these two had non-specific neurological symptoms; the other was asymptomatic. Of the six cases reported overall, five were homozygous for a single nonsense mutation, (c.139C > T, p.Arg47Ter, p.R47*) in *MCEE*, implying a complete loss of MCE activity, and showed moderately decreased propionate incorporation in cultured fibroblasts. The clinical significance of this rare disorder requires clarification.

We describe a new case of MCE deficiency, which contributes to the clinical, biochemical and molecular characterisation of this condition.

2. Patient description

The proband is a French-Canadian female, born at term from an uncomplicated pregnancy. Parents were not consanguineous and the family history was unremarkable. At the age of 5 years, following a previously unremarkable medical history, she presented acutely with vomiting, dehydration, confusion and visual hallucination. There was severe metabolic acidosis with increased anion gap (pH = 6.96, bicarbonate 3.5 mmol/L and pCO_2 15.9 mm Hg, anion gap 25.5 mmol/L), accompanied by mild hyperammonemia (107 μmol/L; ref. < 50), elevated creatine kinase (from 498 IU/L initially up to 3062 IU/L, ref. < 150), and strong ketosis and ketonuria. There was no hyperlactatemia.

Biochemical testing at this time suggested a disorder in the pathway of propionyl-coA metabolism, as described below in Section 3.

Following the initial acute episode, she was treated with carnitine supplementation (100 mg/kg/day) and a protein restricted diet. Subsequently, the protein intake was gradually increased to a near-normal diet, only avoiding excessive protein intake. During intercurrent illnesses, the patient was treated with protein restriction, increased caloric intake and other basic measures. Now at age 11 years, growth and development are completely normal. There have been no further episodes of significant metabolic acidosis. However, at the age of 9 years, she manifested one episode of acute and transient diplopia, which occurred in the context of a fever and lasted about a week.

3. Biochemical investigations and results

3.1. Metabolite profiles in body fluids

Organic acid profiles were analysed by gas chromatography–mass spectrometry following trimethylsilyl derivatisation, using selective ion monitoring for a panel of 58 metabolites. The related method for dedicated quantitation of methylmalonic acid in serum included stable isotope dilution. Serum acylcarnitine profiles were analysed by tandem mass spectrometry with multiple reaction monitoring, following derivatisation by butylation.

During the patient's acute presentation, the organic acid profiles in urine, serum and cerebrospinal fluid (CSF) were dominated by massive elevations of ketones. The urine profile was complex, including marked elevations of many metabolites considered secondary to the acute catabolic state. The serum and CSF profiles were less complex. Table 1 presents the results for methylmalonic acid (MMA) and for several by-products of propionyl-coA, namely methylcitric acid, propionylglycine and 3-hydroxypropionic acid (tiglylglycine was not analysed as part of the organic acid profile) which were observed in samples collected during the acute episode. During subsequent follow-up, with the patient in a stable condition, the organic acid profiles were always unremarkable except for elevations of MMA, methylcitric acid and 3-hydroxypropionic acid. The results obtained over a six-year period of follow-up are also summarised in Table 1.

Acylglycine analysis of the two most recent urine samples, using a sensitive LC–MS/MS method, showed unremarkable profiles, including essentially normal results for propionylglycine and tiglylglycine (data not shown).

The serum acylcarnitine profile during the acute episode showed a marked elevation of C3 (propionyl) carnitine: 21.6 μmol/L (reference range < 0.58). C5:1 carnitine, representing the sum of tiglylcarnitine and methylcrotonylcarnitine, was moderately elevated: 0.19 μmol/L (ref. < 0.05); this was presumed to reflect increased tiglylcarnitine. C4-OH carnitine (the sum of 3-hydroxybutyrylcarnitine and 3-hydroxyisobutyrylcarnitine) was elevated: 2.13 μmol/L (ref. < 0.19), reflecting the observed ketosis. The acute profile showed some other mild elevations, considered secondary to the catabolic state. However, C4DC carnitine, representing the sum of methylmalonylcarnitine and succinylcarnitine, was well within reference range: 0.05 μmol/L (ref. < 0.19). During the six-year follow-up, seven further serum acylcarnitine profiles showed a persistent moderate elevation of C3 carnitine, ranging from 1.53 to 5.82 μmol/L (median, 3.96; ref. < 0.58). The acylcarnitine profiles during follow-up were otherwise unremarkable: there was sometimes a mild elevation of free carnitine, attributable to

Table 1
Concentrations of organic acids relevant to metabolism of propionyl-coA and methylmalonyl-coA, in urine, serum and cerebrospinal fluid, at the time of acute presentation and during long-term follow-up.

	During the acute episode	During follow-up		Reference range:
		Median	Observed range	Upper limit
Organic acids in urine (mmol/mol creatinine)				
Methylmalonate	53	78	47–151	10
Methylcitrate	83	29	15–61	11
3-hydroxypropionate	7310	150	26–418	59
Propionylglycine	24	0	0	0
Organic acids in serum (μmol/L)				
Methylmalonate	48	9	na	1
Methylcitrate	6	1	na	2
3-hydroxypropionate	498	76	na	11
Propionylglycine	0	0	na	0
Organic acids in CSF (μmol/L)				
Methylmalonate	42	nd	na	0
Methylcitrate	32	nd	na	0
3-hydroxypropionate	114	nd	na	1
Propionylglycine	0	nd	na	0
Methylmalonate in serum, dedicated assay (μmol/L)	nd	5.16	3.63–8.35	0.50

CSF, cerebrospinal fluid; nd, not determined; na, not applicable. Observed ranges and median values during follow-up are derived from 9 independent analyses of the urine organic acid profile and 4 independent assays of methylmalonic acid in serum.

dietary supplementation, and occasional mild elevations of a few other acylcarnitines considered non-specific and non-significant. C5:1 carnitine was persistently within reference range during follow-up. Notably, C4DC carnitine always remained within reference range.

Plasma total homocysteine and serum cobalamin were not measured at the time of the acute episode. During follow-up, both were within reference range.

3.2. Interpretation and differential diagnosis

The initial impression, at the time of the patient's acute presentation, was of a likely propionic acidemia, *i.e.* a primary deficiency of propionyl-coA carboxylase (PCC). This working diagnosis was based chiefly on the first urine organic acid profile and the first serum acylcarnitine profile, and the clinical presentation. The serum acylcarnitine profile, with substantial elevation of C3 carnitine, moderate elevation of C5:1 carnitine and normal C4DC carnitine, was typical of profiles seen in propionic acidemia patients; whereas patients with severe methylmalonic acidemia due to defects in methylmalonyl-coA mutase often show elevation of C4DC, reflecting increased methylmalonylcarnitine, particularly during episodes of acute decompensation.

In urine, the propionyl-coA metabolites methylcitric acid, propionylglycine and 3-hydroxypropionic acid were all clearly elevated. Although there was also a mild increase of methylmalonic acid (MMA) in urine, its fold-elevation was less than that of any of the propionyl-coA metabolites. While one would not *a priori* expect patients with propionic acidemia to show any elevation of MMA in urine, within the context of an acute clinical state and an overall complex profile, the significance of such a mild elevation of MMA was not immediately obvious. Such mild elevations of MMA in urine are often seen as secondary findings in various contexts including disturbances of gastro-intestinal structure or function and states of nutritional deficiency, including decreased intake of vitamin B12 (cobalamin) [10]. It is also worth noting, however, that 3-hydroxypropionic acid, which was much more dramatically elevated than the other propionyl-coA metabolites, can also be elevated due to several causes unrelated to inherited metabolic disorders, including production by gut bacteria [10].

In the serum and CSF samples collected during the acute episode, however, the overall profiles were less complex than in urine and the relative elevations of MMA were more striking than in the urine; the potential diagnostic significance of the MMA therefore could not be readily dismissed.

During follow-up, urine MMA remained persistently elevated, but the elevations were always relatively mild. Methylcitric acid likewise remained persistently but mildly elevated. The same was true of 3-hydroxypropionic acid, in contrast to its striking elevation during the initial episode of acute decompensation. Propionylglycine was never again detectable in urine after the initial episode. In serum, MMA was persistently elevated. The normal results for serum cobalamin and total homocysteine argued against a nutritional deficiency of vitamin B12 (cobalamin) and also against most defects of cobalamin uptake, transport or metabolism.

Considering together all the biochemical findings at the time of acute decompensation and during long-term follow-up, the overall biochemical profile was not typical of a defect acting at the level of PCC; either a primary propionic acidemia or a deficiency of multiple carboxylases due to defective biotin metabolism. Nor, however, was it typical of a disorder at the level of methylmalonyl-coA mutase (MUT); either a primary deficiency of this enzyme or a defect related to its cofactor, cobalamin. While there was clearly evidence implying a disorder acting within the propionyl-coA metabolic pathway (Fig. 1), the overall impression was of an "intermediate" biochemical phenotype perhaps reflecting a block at the intervening step between PCC and MUT. We thus came to suspect a deficiency of methylmalonyl-coA epimerase (MCE) as the most likely underlying cause.

As there was no suitable MCE enzyme assay available for direct application to patient cells, two lines of investigation were pursued: molecular analysis of the *MCEE* gene and studies on cultured fibroblasts. The three aims of the fibroblast studies were: to seek confirmation of diminished capacity for flux through the propionyl-coA metabolic pathway; to test the hypothesis of primary MCE deficiency; and to rule out the possibility of diminished activity of PCC or MUT.

3.3. Studies on cultured fibroblasts

Assay of PCC enzymatic activity on the patient's fibroblasts gave results within the reference range: 811 pmol/min/mg protein (control mean 1123, range 295–1837). Moreover, no mutations were detected in the *PCCA* or *PCCB* genes encoding the subunits of PCC. Activities of other biotin-dependent enzymes, pyruvate carboxylase and 3-methylcrotonyl-coA carboxylase, were also normal in fibroblasts (data not shown). The results of these tests (performed in the laboratory of Dr. M. Ugarte, Universidad Autonoma de Madrid) effectively ruled out propionic acidemia and related disorders of biotin metabolism.

The synthesis of cobalamin coenzyme derivatives from [^{57}Co]CNCbl (assay method described in [11]) yielded results within reference ranges. Synthesis of adenosylcobalamin by the patient's fibroblasts was 9.4% of total (control mean, 15.3%, standard deviation 4.2), while synthesis of methylcobalamin was 60.0% (control mean 58.0%, SD 6.7).

Incorporation of [^{14}C]propionate [11] was somewhat lower than control values, and did not respond to the presence of hydroxycobalamin in the culture medium. In absence of hydroxycobalamin, the patient's cells incorporated 4.1 nmol propionate/mg protein/18 h (control mean 10.8, SD 3.7); in presence of hydroxycobalamin, the patient's cells incorporated 3.3 nmol propionate/mg protein/18 h (control mean 10.9, SD 3.5).

The moderately decreased propionate incorporation was confirmed in two successive experiments, in which complementation studies [11] were also performed. Results are shown in Table 2.

Fusion of cultured fibroblasts from the patient with cells of the *mut*, *cblA* and *cblB* complementation classes respectively brought about an increase in propionate incorporation, indicating that the patient did not belong to any of those classes. However, fusion of cells from the patient with cells from each of two known patients in the *MCEE* complementation class did not cause any increase in propionate incorporation.

Table 2
Results of cellular complementation analysis.

Cell lines included in assay	Incorporation of propionate (nmol/mg protein/18 h):		
	Cell line prior to complementation	Complementation without cell fusion (− PEG)	Complementation with cell fusion (+ PEG)
Experiment 1:			
Patient alone	3.3	na	na
cblA	1.3	2.7	7.1
cblB	0.9	2.9	11.1
mut (1)	0.8	2.7	10.3
mut (2)	1.0	3.2	7.0
Experiment 2:			
Patient alone	4.2	na	na
mut (1)	0.6	2.1	5.6
MCEE (1)	4.3	4.6	4.1
MCEE (2)	2.9	3.2	2.9
MCEE (1) + *MCEE* (2)[a]	(as above)	2.4	2.2

Equal numbers of fibroblasts from the patient being tested and fibroblasts from various known complementation classes were mixed and fused by exposure to 40% (w/v) polyethylene glycol (PEG). Propionate incorporation was compared in parallel fused and unfused cultures. Incorporation is increased in mixed fused cultures from different complementation classes compared to parallel mixed unfused cultures. Cells from a given patient complement cells from every complementation class except the class that the patient belongs to. The *MCEE* cell lines included in Experiment 2 are from two previously reported patients; *MCEE* (1) from [6] and *MCEE* (2) from [5]. [a]Fusion of these two *MCEE* cell lines with each other, without inclusion of the Patient cell line, was performed as an internal control. na, not applicable.

This indicated that this patient belongs to the *MCEE* class, *i.e.* that she is deficient in activity of methylmalonyl-coA epimerase, MCE.

4. Molecular investigations of *MCEE* and results

Extracted DNA from blood was sequenced to screen for mutations in the three coding exons and splice site junctions of *MCEE*, by the Sanger method using a 3730XL DNA analyzer (Applied Biosystems), at the McGill University and Genome Quebec Innovation Center. A heterozygous variant (c.139C > T, p.Arg47Ter, Refseq NM_032601.3) was found in exon 2 (Fig. 2). This mutation was previously reported in the homozygous state in several unrelated patients with MCE deficiency, as described above in the Introduction. Deletion/duplication analysis by exon array (MitoMet V3.0 oligonucleotide microarray, performed by BCM Medical Genetics Laboratories) yielded normal results.

We then searched for an intronic sequence variation that could result in an aberrant splicing of the transcript. Total RNA was extracted from whole blood and reverse transcribed to cDNA using a standard protocol (SuperScript® VILO cDNA Synthesis Kit, Life Technologies). PCR-based amplification of *MCEE* transcript isoforms was performed, as previously described, at the RNomics platform of Université de Sherbrooke [12]. This showed expression of an additional transcript that included a 92 bp sequence between exons 2 and 3, compared to control (Fig. 2). The splicing index of this alternate transcript suggests that its abundance is equal to that of the wild type transcript. cDNA sequencing of this alternative transcript confirmed that the additional 92 bp sequence mapped to intron 2 (genomic position hg19 chr2: 71,337,897–71,337,988). This sequence corresponds to a putative 96 bp untranslated exon in a known spliced EST (CA427623), but missing the last 4 nucleotides at the 3′ end. DNA sequencing of the 3′ end of this putative untranslated exon showed a heterozygous variation (c.379-644 A > G, genomic position hg19 ch2: 71,337,896) creating a new consensus donor splice site GTAA (Fig. 2). All the three alternative reading frames result in a premature stop codon. This variation was not reported previously in the dbSNP database. There is no sequencing coverage in this region for the ExAC or EVS databases to allow an estimation of population frequency.

Sequencing of the mother's DNA revealed that the intronic variation c.379-644 A > G was maternally inherited. Although DNA from the father was not available, the mutation c.139C > T (p.Arg47Ter) was absent in the mother's DNA, and was thus presumed to be paternally inherited.

5. Discussion

We describe a new patient with deficiency of methylmalonyl-coA epimerase. While several previously reported MCE deficiency patients did not present with an acute metabolic decompensation or with acidosis, and the clinical significance of this biochemical disorder has remained unclear, our patient is now the second recognised with an acute metabolic presentation. MCE deficiency should therefore be considered as a condition with potentially significant clinical implications. Following the initial episode our patient subsequently has not required aggressive ongoing management to maintain normal development, and long-term clinical course remains relatively uneventful on a essentially normal diet. The severity of the initial presentation led us to maintain a careful approach by treating undercurrent illness with caloric supplementation and protein restriction. While there is very little data available on similar cases, we advise prudence for clinicians encountering patients with this disorder.

Five of the six cases described in previously published articles were homozygous for the nonsense mutation c.139C > T, p.Arg47Ter. Comparing the observed propionate incorporation in fibroblasts from our patient with data from those five patients, the degree of diminution of incorporation is similar. The splicing mutation identified in our patient is predicted to result in a null mutation, as the incorporation of the intronic sequence between exon 2 and 3 would result in a premature stop codon. In accordance with this, results from our *in vitro* method suggest that the relative abundance of this alternate transcript

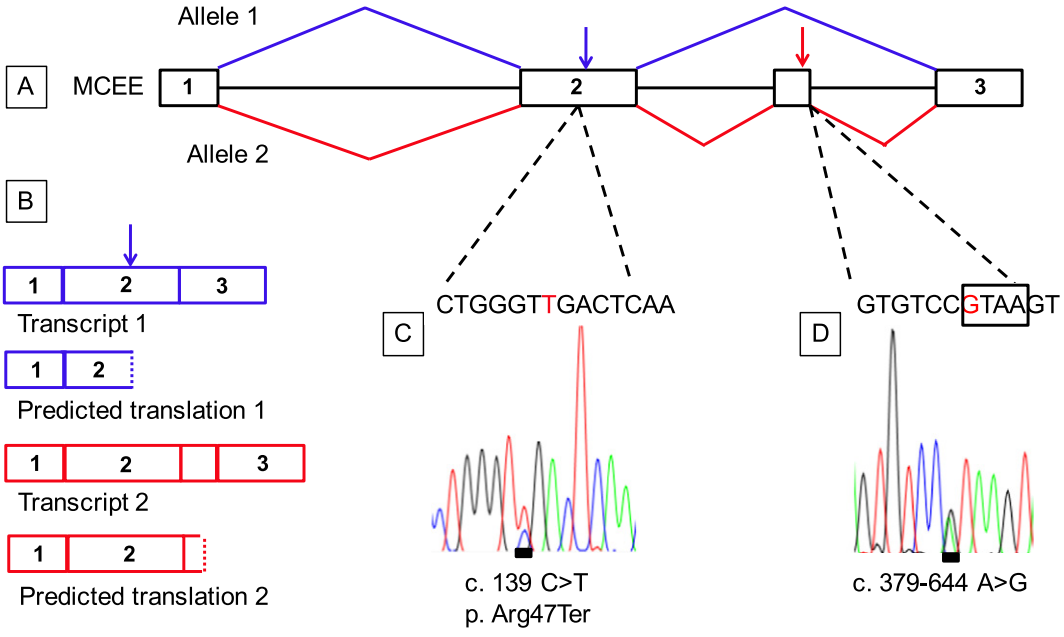

Fig. 2. *MCEE* mutations in the affected patient. The three coding exons are numbered and an additional 96 bp putative untranslated exon is shown between exons 2 and 3. (A) Normal splicing is shown (in blue). Aberrant splicing (shown in red) is caused by a mutation located 4 bp preceding the 3′ end of the untranslated exon, which creates a strong consensus splice site resulting in a 92 bp fragment inserted between exons 2 and 3. The positions of the two mutations identified in the patients are localised by arrows. (B) Gene products: two abnormal transcripts identified in the patient. The predicted translation of each is also shown. (C) The mutation p.Arg47Ter was validated by Sanger sequencing. It causes a premature stop codon. (D) The mutation c.379–644 A > G was validated by Sanger sequencing. It causes an aberrant splicing.

containing the intronic insertion is ~50% of total, with the other allele likewise accounting for ~50%. We cannot exclude the possibility that up to 5% of wild type transcript could be produced by the intronic mutation allele, permitting some residual MCE enzyme activity and possibly contributing to a relatively late-onset presentation. However, it is also important to note that, even in the presence of homozygosity for an *MCEE* nonsense mutation, there is some residual propionate incorporation. This is presumably due to the existence of alternative shunt pathways allowing some conversion of propionyl-coA to succinyl-coA in the situation where there is no MCE activity. This provides a plausible explanation why complete MCE deficiency is clinically less severe than the classic forms of propionic acidemia and methylmalonic acidemia.

The sixth previously reported case of MCE deficiency was homozygous for a missense mutation (c.178 A > C,p.K60Q, p.Lys60Gln). In that case, there was a clear elevation of methylmalonic acid in urine, but observed propionate incorporation in fibroblasts was within reference range. This patient was described as having deterioration of motor function, ataxia, mild spastic paraplegia and dysarthria [9]. This sequence variant has been classified as "pathogenic" in the Clinvar database, mainly based on that single brief report. However, results of *in silico* analysis are conflicting. According to SIFT, it is tolerated, and using Polyphen 2 it is considered benign, whereas with PROVEAN it is considered deleterious. Notably, 8 homozygotes are reported in the ExAC database (~60,000 individuals) and allele frequency is 1.5% in south Asians. This population-based data tends to argue against pathogenicity, or at least to imply that this mutation would give rise to a very mild condition with normal survival and reproduction rate. Nonetheless, one cannot exclude the possibility that partial MCE deficiency could be a factor putting individuals at increased risk of neurologic disease, but with incomplete penetrance.

There is currently no MCE enzyme assay available which would be directly applicable to human cells in the clinical setting. Direct assays of the recombinant human enzyme and of recombinant MCE enzymes from other species have been described [13–15], providing a possible option for *in vitro* expression analysis of missense mutant MCE proteins if required, although these methods require the isolation of partially purified proteins.

Our patient is the first to be described who is compound heterozygous for two different mutations in *MCEE* rather than homozygous for a single mutation. In the previously reported study of a series of cell lines from patients with methylmalonic aciduria [9], there were two in which only a single heterozygous *MCEE* sequence variation (c.427C > T, p.R143C, p.Arg143Cys in both cases) was found by sequencing of exons. We have now tested both of those cell lines for the novel intronic splicing mutation identified in our patient; with negative results. The significance, if any, of the variation found in those two cases thus remains unclear, particularly considering that propionate incorporation was within reference range in both heterozygous cell lines. *In silico* predictions for this variant are as follows: Polyphen2, probably damaging; PROVEAN, deleterious; SIFT, damaging. However, in the ExAC dataset: 10 homozygotes are reported, and the allele frequency in Finnish populations is 2.6%. This population data leads to a similar interpretation as for the variant p.Lys60Gln. While we cannot exclude the possibility that simple heterozygosity for certain *MCEE* mutations could cause mild elevation of MMA in urine or blood under certain conditions, for example by a dominant negative effect, there is no existing positive evidence to support such a phenomenon. Another remote possibility could be that heterozygosity for an *MCEE* mutation together with a heterozygous mutation at another locus relevant to MMA metabolism could cause elevation of MMA in body fluids. In the meantime, however, these two heterozygous p.Arg143Cys cases remain unclassified and they do not contribute to characterisation of the MCE deficiency clinical or biochemical phenotype. Conversely, it is also possible that some missense mutations allowing significant residual activity of MCE might not cause methylmalonic aciduria, even in homozygosity or compound heterozygosity, thus contributing to the apparent rarity of this condition.

In addition to the six cases of MCE deficiency previously reported, and the patient described in our present article, the identification of a further nine cases was indicated in an abstract [16]. These patients were identified by sequencing of *MCEE* in DNA from 150 fibroblast cell lines banked from patients with mild or moderate methylmalonic aciduria. Eight of these cases were homozygous for the same previously-described nonsense mutation, p.Arg47Ter, while the ninth was homozygous for a novel missense mutation. However, no clinical description of cases was included in that abstract. Further characterisation of this group would contribute to understanding of this apparently rare condition. One other abstract [17] briefly describes a single patient, homozygous for p.Arg47Ter, who presented with an episode of acute metabolic acidosis.

The initial working diagnosis for our patient, at the time of her acute presentation, was propionic acidemia. We previously flagged this potential pitfall in a preliminary abstract [18], and now provide a detailed description of the biochemical phenotype, its evolution during long-term follow-up and the further biochemical and molecular investigations which were needed to confirm the true diagnosis of MCE deficiency for this patient. Another recent abstract [19] refers to a patient originally suspected to have propionic acidemia but for whom subsequent next-generation gene panel analysis instead revealed homozygosity for the p.Arg47Ter mutation in *MCEE*. Such reports suggest that MCE deficiency might thus far be underdiagnosed, particularly considering that the associated MMA elevations are often relatively mild even in patients with genotypes predicting complete loss of MCE activity. On the other hand, the overall allele frequency of p.Arg47Ter in the ExAC database is only 0.025% (0.042% in Europeans), with no homozygotes listed. These data are compatible with the apparent rarity of this condition.

The organic acid profiles of our patient were in fact not typical of propionic acidemia, nor of classical methylmalonic acidemias, nor of other 'non-classical' methylmalonic acidemias such as SUCLA or SUCLG disorders. It is possible that the observed disproportionate elevation of 3-hydroxypropionic acid could be a particular feature of MCE deficiency, as in this situation 3-hydroxypropionic acid might potentially be formed by alternative metabolism of the accumulating D-methylmalonyl-coA and/or of free methylmalonic acid. This would represent a route different from the pathway by which 3-hydroxypropionic acid is generated from propionyl-coA in propionic acidemia [20]. Certain aspects of our patient's organic acid profiles during decompensation, namely ketoacidosis with marked elevation of 3-hydroxypropionic acid, considerable elevation of methylcitric acid and propionylglycine, and relatively mild elevation of MMA, together may represent a characteristic pattern to be seen in other patients with MCE deficiency.

In summary, we have identified and characterised a new case of the rare disorder MCE deficiency, presenting with acute metabolic decompensation. We have described the biochemical phenotype of this patient, highlighting a possibility of diagnostic confusion in the context of an initial acute presentation, and emphasising that the observation of persistent mild or moderate methylmalonic aciduria is a clue which warrants consideration of MCE deficiency in the differential diagnosis. The finding in this case of a novel intronic splicing mutation in *MCEE* also shows that sequencing of exons alone is not sufficient to diagnose this condition in all cases, or to exclude it.

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