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BRIEF REPORT

Monocarboxylate Transporter 1 Deficiency and Ketone Utilization

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SUMMARY

Ketoacidosis is a potentially lethal condition caused by the imbalance between hepatic production and extrahepatic utilization of ketone bodies. We performed exome sequencing in a patient with recurrent, severe ketoacidosis and identified a homozygous frameshift mutation in the gene encoding monocarboxylate transporter 1 (*SLC16A1*, also called *MCT1*). Genetic analysis in 96 patients suspected of having ketolytic defects yielded seven additional inactivating mutations in *MCT1*, both homozygous and heterozygous. Mutational status was found to be correlated with ketoacidosis severity, MCT1 protein levels, and transport capacity. Thus, MCT1 deficiency is a novel cause of profound ketoacidosis; the present work suggests that MCT1-mediated ketone-body transport is needed to maintain acid–base balance.

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Acetoacetate and 3-hydroxybutyrate are slightly acidic biomolecules that, together with acetone, are called ketone bodies and serve as the major circulating energy source during fasting. Ketone bodies are formed in the liver from the ultimate breakdown product of fatty acids — acetyl coenzyme A (CoA) — by coupling of two acetyl units in a three-step enzymatic process called ketogenesis. Ketone bodies are believed to undergo passive distribution to metabolically active tissues, where they are used as an energy source.[1]

Ketoacidosis, a pathologic state, occurs when ketone formation exceeds ketone utilization. The clinical consequences of ketoacidosis are exemplified by diabetic ketoacidosis, a condition that is marked by vomiting, osmotic diuresis, dehydration, and Kussmaul breathing and that may progress to decreased consciousness and, ultimately, death.[2]

Inborn errors of ketone utilization are manifested similarly; however, glucose levels in these types of ketoacidosis are normal or even low, in contrast to glucose levels in diabetic ketoacidosis.[3] Only two genetic causes of recurrent ketoacidosis are currently known: succinyl CoA oxoacid transferase (SCOT) deficiency (Online Mendelian Inheritance in Man [OMIM] database number, 245050) and mitochondrial acetoacetyl-CoA thiolase (ACAT1) deficiency (also called beta-ketothiolase deficiency; OMIM number, 203750). Both SCOT and ACAT1 are involved in ketolysis, the breakdown of ketone bodies into the key cellular energy source, acetyl CoA.

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We performed targeted exome sequencing of homozygous genomic regions in a patient of consanguineous descent who had recurrent, severe ketoacidosis. A homozygous mutation was detected in the gene encoding monocarboxylate transporter 1 (*MCT1*). Subsequently, we evaluated a series of 96 patients with recurrent ketoacidosis, in whom known ketolytic defects had been ruled out enzymatically, to identify additional patients with mutations in *MCT1* or related genes.

METHODS

STUDY PARTICIPANTS AND GENETIC STUDIES

We performed targeted exome sequencing of homozygous genomic regions in the index patient and her family members. Regions of homozygosity in the index patient were determined with the use of a high-resolution single-nucleotide polymorphism (SNP) array. Coding parts of homozygous regions were then captured on a custom array and sequenced as described elsewhere.[4,5] To follow up on the findings from exome sequencing, we sequenced the entire coding region of *MCT1* in a series of 96 patients with ketoacidosis in whom known ketolytic defects had been ruled out because of the normal enzymatic activities of ACAT1 and SCOT. In addition, we performed Sanger sequencing of the related genes *MCT2* (*SLC16A7*), *MCT3* (*SLC16A8*), and *MCT4* (*SLC16A3*), plus the ancillary gene *BSG*, in these patients. Heterozygosity of mutations was confirmed by means of complementary DNA (cDNA) sequencing and genomic deletion analysis.

Further details of sequencing and analysis are available in the Supplementary Appendix, available with the full text of this article at NEJM.org. Written informed consent for targeted whole-exome sequencing and Sanger sequencing was obtained from the parents of the index patient for themselves, the index patient, and her two siblings. Sanger sequencing of candidate genes in the cohort of patients with a suspected ketolytic defect and blood collection for functional studies were performed as a part of the diagnostic process (approved by the medical ethics committee of the University Medical Center Utrecht).

MCT1 EXPRESSION AND FUNCTIONAL STUDIES

Erythrocyte lactate transport was measured essentially as described by Fishbein.[6] For the analysis of each blood sample from a patient, at least one control sample was included in the same experiment, with the same procedures used for blood collection, transport, and analysis. Control blood samples were taken at random from blood left over from diagnostic tests, which was used anonymously in this study. Immunoblotting was performed with fibroblast homogenates and an affinity-purified rabbit polyclonal antibody against MCT1 and MCT4. Fibroblast homogenates from 10 healthy volunteers were also used as controls. Further details on functional and expression studies are available in the Supplementary Appendix. Reference blood results from testing of children before and after 24 hours of fasting were obtained from published data.[7]

STATISTICAL ANALYSIS

All applied statistical tests were two-tailed unpaired t-tests. P values of less than 0.05 were considered to indicate statistical significance.

RESULTS

CASE REPORT

The index patient had repetitive episodes of profound metabolic acidosis, with a blood pH below 7.00 on three occasions, all accompanied by massive urinary excretion of 3-hydroxybutyrate and acetoacetate, with plasma lactate and ammonia levels remaining in the normal range. Between episodes, results of blood gas analyses were normal. See the Supplementary Appendix for an extended case report.

GENETIC STUDIES

SNP array analysis in the index patient revealed 36 homozygous genomic regions larger than 1 Mb, with 7 of the 36 larger than 10 Mb, which confirmed consanguinity. Targeted exome sequencing of coding parts of these homozygous regions in the five family members yielded nine rare variants. Of these, a single-nucleotide insertion disrupting the reading frame of *MCT1* at asparagine 15 (c.41dupC; National Center for Biotechnology Information reference sequence number, NM_001166496.1) was the strongest candidate. The correct segregation of this mutation in the family was confirmed by means of Sanger sequencing. This variant was absent from multiple large variation databases (1000 Genomes, dbSNP, and the National Heart, Lung, and Blood Institute [NHLBI] GO Exome Sequencing Project Exome Variant Server [data release ESP6500]). The insertion of an extra nucleotide early in the gene se-

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quence results in a frameshift and thus in a loss of MCT1 function.

MCT1 MUTATIONS IN ADDITIONAL PATIENTS WITH KETOACIDOSIS

MCT1 is one of the transmembrane transporters encoded by members of the SLC16 gene family; among these transmembrane transporters, MCT1, MCT2, MCT3, and MCT4 have been shown to transport monocarboxylates, including lactate and ketone bodies.[8] MCT1 requires the glycoprotein BSG (also called CD147) for proper subcellular expression.[9,10] We therefore sequenced *MCT1* and related genes in a series of 96 patients with unexplained ketoacidosis. We identified seven additional suspected deleterious variants, all in *MCT1*, including six truncating mutations and one missense mutation (Fig. 1A and Table 1). All truncating mutations are expected to result in a loss of protein function. The missense mutation, p.Arg313Gln, targets a highly conserved residue that is essential for catalytic activity.[11] In total, we identified 9 patients with mutations in *MCT1*, 3 homozygous and 6 heterozygous, including 2 siblings. None of these mutations were seen previously in population-based variation databases (1000 Genomes, dbSNP, NHLBI GO Genome Sequencing Project Exome Variant Server, and Genome of the Netherlands).[12] Together, the genetic data firmly link defects in *MCT1* to ketoacidosis in our patients.

MCT1 EXPRESSION

Next, we studied the effect of the mutations in *MCT1* at the protein level. Immunoblot analysis with fibroblast homogenates from patients with a homozygous *MCT1* mutation, performed with antibodies raised against a C-terminal peptide of MCT1, confirmed that predicted truncating mutations lead to an absence of the full-length protein (Fig. S1A in the Supplementary Appendix). In five patients, we could detect only a single heterozygous truncating mutation in *MCT1* (Fig. 1A), despite sequencing the complete coding region. Analysis of cDNA confirmed biallelic expression (Fig. 1B). A custom multiplex amplification quantification analysis further ruled out the presence of exon-sized deletions in heterozygous patients. Immunoblot analysis showed significantly reduced levels of MCT1 protein in fibroblasts from patients with a heterozygous truncating mutation, relative to levels in controls (Fig. 1C, and Fig S1B in the Supplementary Appendix). The immunoblot results confirmed that there was an MCT1 deficiency at the protein level. We tested fibroblasts to determine whether the MCT1 deficiency was compensated for by increased cellular expression of MCT4 but found no evidence for this (Fig S1C in the Supplementary Appendix).

MONOCARBOXYLATE TRANSPORT ASSAY

Next, we studied the effect of mutations in *MCT1* on monocarboxylate transport, using a previously described assay for MCT1 activity that measures lactate efflux from erythrocytes endogenously loaded with lactate.[6,13] In the samples from patients with a homozygous mutation in *MCT1*, erythrocyte lactate transport was significantly reduced as compared with that in the control samples (Fig. 1D). The mean lactate transport activity in erythrocytes from heterozygous carriers, both symptomatic and asymptomatic, was significantly reduced as compared with the transport activity in control samples but was significantly higher than that in homozygous patients (Fig. 1D).

CLINICAL SYMPTOMS

All patients presented with bouts of ketoacidosis provoked by fasting or infections in their first years of life (Table 1), as is illustrated for the index patient in Figure 2A, and Figure S2A in the Supplementary Appendix. The pH of the blood was normal between episodes. Excretion of urinary ketones under these circumstances ranged from normal to clearly elevated (Fig. 2B). Ketoacidotic episodes were preceded by poor feeding and vomiting and were associated with dehydration, which was a consequence of osmotic diuresis and vomiting. Hypoglycemia was observed infrequently, and glucose levels usually remained in the normal range (Fig. S2B and S2C in the Supplementary Appendix). In some patients, repetitive vomiting led to a diagnosis of ketotic vomiting or abdominal migraine. Profound metabolic acidosis was associated with decreased consciousness and insufficient respiratory drive (Fig. 2C), which worsened the degree of acidosis.

In all patients, treatment with intravenous glucose or dextrose (combined with bicarbonate) led to rapid clearance of metabolic acidosis. Early initiation of treatment appeared to prevent ketoacidosis. Similarly, ensuring an adequate caloric intake reduced the number of episodes. The frequency of ketoacidotic episodes appeared to de-

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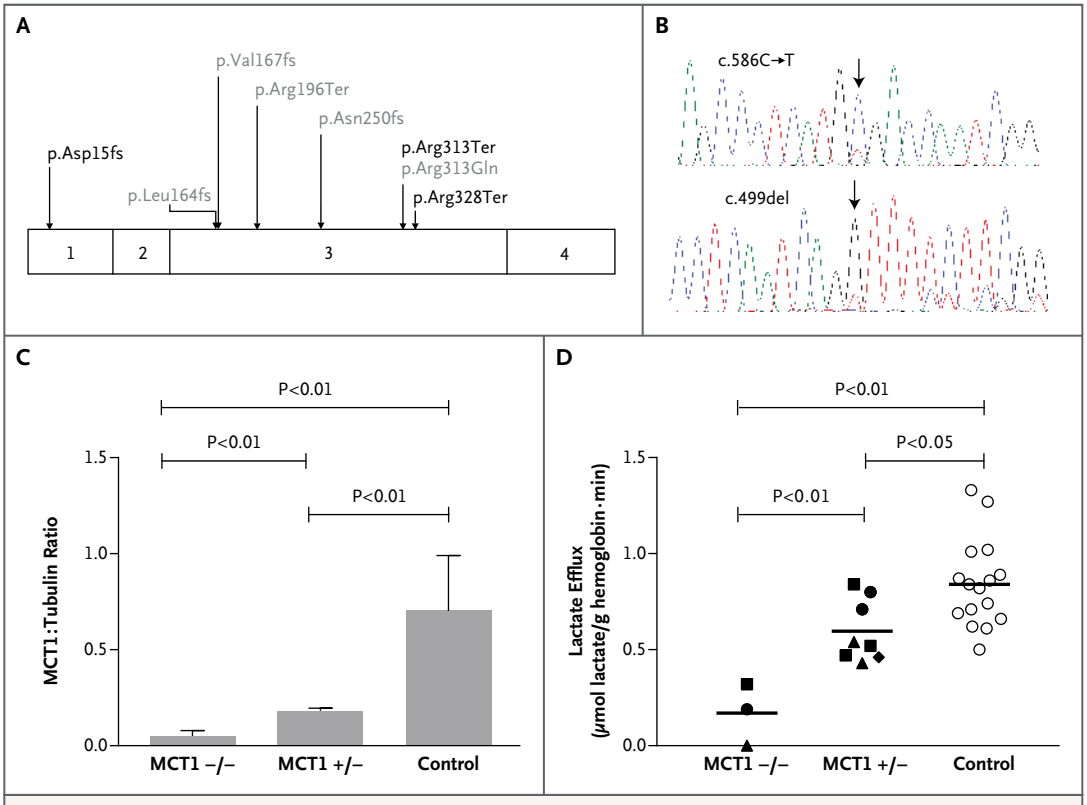


Figure 1. MCT1 Deficiency in Patients with Episodes of Ketoacidosis.

Panel A shows the identified mutations in the monocarboxylate transporter 1 (MCT1) protein, with the four different exons indicated. The full-length MCT1 protein consists of 500 amino acids. Heterozygous mutations are indicated in gray, and homozygous mutations in black. Panel B shows examples of the sequencing of complementary DNA in fibroblasts from patients with heterozygous mutations; the colors represent bases: green denotes A, red T, black G, and blue C. The arrows indicate the mutated residue. The majority of the transcript is unmutated MCT1 sequence, and the minority is the mutated sequence as a result of nonsense-mediated decay. Panel C shows the MCT1:tubulin ratio from a quantitative immunoblot with fibroblast homogenates from 4 patients with heterozygous truncating mutations as compared with 10 healthy controls. Panel D shows lactate efflux from erythrocytes endogenously loaded with lactate. Efflux was measured for patients with a homozygous *MCT1* mutation and their heterozygous family members (solid circles denote p.Arg313Ter, squares p.Arg328Ter, and triangles p.Asp15fs), a heterozygous patient (p.Arg313Gln, denoted by the diamond), and controls (denoted by open circles). The lactate transport in the homozygous patients was significantly lower than in both controls and heterozygotes. The mean lactate transport activity in heterozygotes was also reduced. Horizontal bars indicate the mean transport activity.

crease over time, and none of the patients had documented ketoacidosis after 7 years of age. Nevertheless, some patients continued to have marked ketonuria associated with (mild) infections.

Patients with homozygous mutations in *MCT1* tended to present at a younger age ($P=0.05$) and had more profound ketoacidosis (Fig. 2D, and Fig. S2D in the Supplementary Appendix). In addition, homozygous patients had mild-to-moderate developmental delay, whereas patients with milder deficiencies of *MCT1* had normal development.

DISCUSSION

We identified MCT1 deficiency as a disorder of ketone utilization, expanding the spectrum of disorders leading to ketoacidosis from those of ketolysis to those of ketone delivery. This finding may aid in timely diagnosis of the disorder and allow for improved disease management. In addition, this observation implies that MCT1-mediated import of ketone bodies into extrahepatic tissues is essential during periods of catabolic stress in order to maintain acid–base balance.

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Table 1. Spectrum of *MCT1* Mutations and Associated Phenotypes.*

Variant	Effect	Inheritance	Phenotype	Patient Characteristic						
				Ancestry	Parental Relatedness	Sex	No. of Ketotic Events	Age at Diagnosis/Last Event/Last Follow-up	Intellectual Disability	Other Clinical Features
Homozygous										
c.41dupC	p.Asp15fs	Sporadic	Profound ketoacidosis	Syrian	Second-degree cousins	F	5	3 mo/7 yr 5 mo/ 8 yr	Moderate	Microcephaly, atrial septal defect, hypoplastic left pulmonary artery and main bronchus
c.937C→T	p.Arg313Ter	Sporadic	Profound ketoacidosis	Irish	None	F	4	1 yr 2 mo/ 7 yr 8 mo/21 yr	Moderate	Epilepsy
c.982C→T	p.Arg328Ter	Sporadic	Profound ketoacidosis	Turkish	Third-degree cousins	M	1	1 yr 11 mo/ 1 yr 11 mo/9 yr	Mild	Cleft palate, coughing attacks during exercise
Heterozygous										
c.586C→T	p.Arg196Ter	Sporadic	Cyclic vomiting, ketoacidosis with massive ketonuria	British	None	M	3	3 yr 11 mo/ 7 yr 6 mo/10 yr	None	Migraine
c.747_750del	p.Asn250fs	Familial	Ketoacidosis with massive ketonuria	British	None	M	10	3 yr 8 mo/ 14 yr/20 yr	None	Migraine
c.747_750del	p.Asn250fs	Familial	Ketoacidosis with massive ketonuria, pregnancy-related vomiting	British	None	F	5	6 yr 3 mo/ 17 yr/22 yr	None	—
c.499del	p.Val167fs	Sporadic	Exaggerated ketotic hypoglycemia, ketoacidosis with massive ketonuria	British	None	F	5	1 yr 6 mo/ 3 yr 9 mo/11 yr	None	—
c.490dupC	p.Leu164fs	Sporadic	Ketoacidosis with massive ketonuria	Dutch	None	M	4	3 yr 2 mo/ 7 yr 5 mo/10 yr	None	Short stature
c.938G→A	p.Arg313Gln†	Sporadic	Cyclic vomiting, ketoacidosis with massive ketonuria	Dutch	None	M	5	2 yr 6 mo/ 6 yr 5 mo/22 yr	None	Exercise intolerance

* The complementary DNA and protein annotations are based on National Center for Biotechnology Information reference sequence numbers NM_001166496.1 and NP_001159968.1, respectively.

† The variant was predicted by PolyPhen-2 to be "probably damaging" and by SIFT to be "deleterious."

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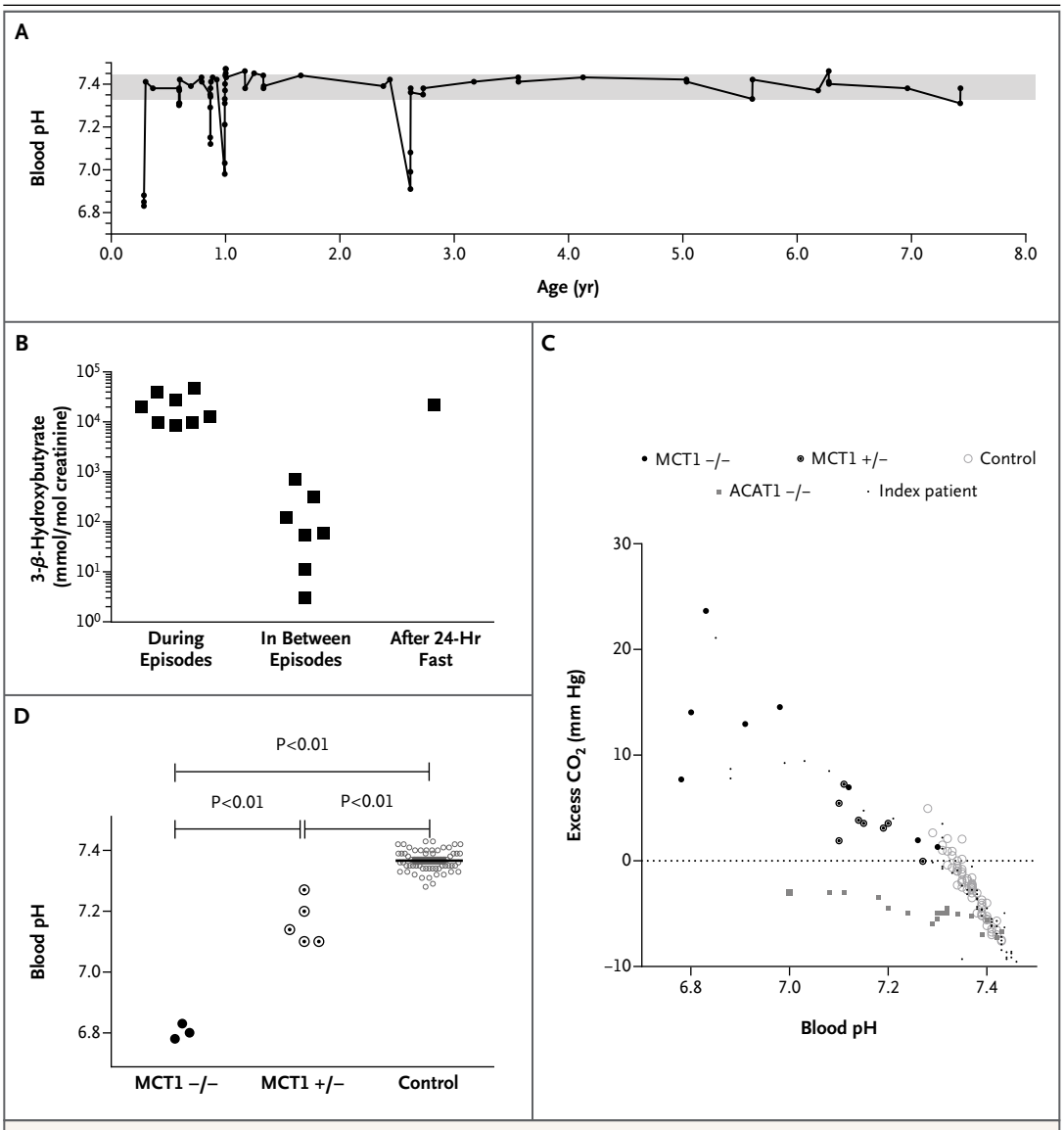


Figure 2. Blood Test Results from Patients with MCT1 Deficiency.

Panel A shows the blood pH over time for the index patient (who had the p.Asp15fs mutation). The ketoacidotic events are clearly visible. An extended case report for this patient is available in the Supplementary Appendix. Panel B shows the 3-β-hydroxybutyrate levels in urine, normalized to creatinine, in three MCT1-deficient patients. Control values are less than 10 mmol per mole of creatinine in between episodes and between 160 and 6400 mmol per mole of creatinine after 24 hours of fasting. Panel C shows the carbon dioxide excess (actual carbon dioxide concentration minus expected carbon dioxide concentration) versus the pH in homozygous or heterozygous MCT1-deficient patients and controls. The expected carbon dioxide concentration was calculated according to Winter's formula; the horizontal dotted line indicates the absence of excess carbon dioxide. Data points for the index patient show clearly that blood values were within the normal range except during ketotic events. Values during a ketotic event in a patient with mitochondrial acetoacetyl-CoA thiolase (ACAT1) deficiency form a separate constellation from the values for MCT1-deficient patients. Panel D shows the lowest recorded blood pH value during a ketotic event in homozygous and heterozygous MCT1-deficient patients and controls. The horizontal bar indicates the mean control value. The pH is lower in the homozygous and heterozygous patients than in controls and lower in homozygotes than in heterozygotes.

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MCT1 encodes a transmembrane protein that facilitates proton-linked transport of a range of monocarboxylate metabolites, such as lactate, pyruvate, and ketone bodies, across the cellular membrane.[8] Our observation of profound ketoacidosis in patients with MCT1 deficiency highlights the fact that facilitated transport of ketone bodies by MCT1 is essential during catabolic stress, when ketone turnover in the body is orders of magnitude higher than in the fed state.

Heterozygous missense mutations in *MCT1* have previously been reported in association with suboptimal erythrocyte lactate transport resulting in muscle injury on exercise and heat exposure.[13] Our patient with a heterozygous missense mutation had exercise intolerance, which indicates a possible overlapping phenotype. Mutations leading to increased expression of MCT1 are associated with exercise-induced hyperinsulinism.[14,15]

Monocarboxylate transporters show tissue-specific variation in expression between species,[16] which underlines the importance of insights gained in our study of *MCT1* function in human disease. Homozygous *MCT1*-knockout mice die early during embryogenesis,[17,18] in contrast to the patients with a complete loss of *MCT1* function in this study. Mouse placenta and human placenta express *MCT1* in opposite subcellular arrangements,[19] which provides a possible explanation for the differences in embryonic lethality. Disruption of *MCT1* in the central nervous system produces axon damage and neuronal loss in mice.[17] The patients with homozygous loss-of-function mutations in *MCT1* described in our study have moderate intellectual disability; however, at this stage it remains unclear whether this is a direct effect of the absence of *MCT1* in the brain or caused by episodes of profound ketoacidosis.

The use of acidic biomolecules as an energy source presents a potential threat to acid–base homeostasis and therefore requires a careful balance between production and consumption. This aim is achieved indirectly, by linking the rate of ketogenesis with cellular energy status in extrahepatic tissues. Concerted action of glucagon and insulin released by the pancreas determines the rate of ketogenesis in the liver. The massive ketoacidosis in patients with MCT1 deficiency and other disorders of ketone utilization — SCOT deficiency and ACAT1 deficiency — unveils the weak spot of this indirect feedback mechanism. In these disorders, the conversion of ketone bodies to acetyl CoA becomes rate limiting. As a consequence, ketone production is uncoupled from consumption, leading to profound metabolic acidosis.

We found that a single heterozygous *MCT1* mutation can result in a deficiency of the transporter and in clinical symptoms. These findings suggest that *MCT1* deficiency is more prevalent than is apparent among previously described disorders of ketone utilization. The heterozygous family members of patients with homozygous *MCT1* loss-of-function mutations had no history of ketoacidosis, which strongly suggests that heterozygous mutations — and possibly even homozygous mutations — in *MCT1* give rise to ketoacidosis only in conjunction with certain genetic and environmental factors. The varying number of episodes and long symptom-free intervals in both heterozygous and homozygous patients support this hypothesis. Future research will have to identify the factors involved in triggering the development of symptoms.

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Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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APPENDIX

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