

CASE REPORT

A founder mutation in the *ETHE1* gene and ethylmalonic encephalopathy in the Omani population

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ABSTRACT

Background: Ethylmalonic encephalopathy (EE) is a devastating early-onset inborn error of metabolism, and heterogenous disorders manifest as chronic diarrhea, petechial rash, and neurological manifestations. The mutation in the *ETHE1* gene leads to hydrogen sulfide accumulation and eventually results in mucosal cell damage in the large intestines and vascular endothelial cells system.

Case presentation: Here, we describe four patients from three different tribes in Oman, and the clinical data revealed that the four patients shared an early-onset phenotype and the neurological manifestations were variable. The biochemical markers, specifically the urine organic acid and hyperlacticemic acidosis, supported and tailored the diagnosis. Molecular diagnosis was confirmed by full gene sequencing of the *ETHE1* gene in the index case and followed by target variant testing for others. Interestingly, all four patients identified to harbor the same homozygous missense pathogenic variant (c.487C >T) in the *ETHE1* gene, and their parents were all heterozygous. These findings indicate that we probably have a founder variant associated with EE in our area.

Conclusion: These findings are of great importance for diagnosis and surveillance for Omani families with EE. Given the relatively high number and frequency of genetic diseases in the region and the limited resources, screening for these founder mutations should provide a rapid and cost-effective tool for molecular diagnosis. Additionally, these findings should help in designing appropriate measures for carrier screening measures at the regional level.

Keywords: Ethylmalonic encephalopathy, Oman, *ETHE1* gene.

Introduction

Ethylmalonic encephalopathy (EE; OMIM #602473) is a rare progressive autosomal recessive inborn error of metabolism caused by a mutation in the *ETHE1* gene that encodes a mitochondrial sulphur dioxygenase involved in the catabolism of hydrogen sulphide (1). EE's clinical presentation is heterogeneous, involving gastrointestinal, neurological, and vascular systems manifesting as chronic diarrhea, failure to thrive, recurrent petechial rash without bleeding diathesis, generalized hypotonia, developmental regression, infantile spasm, orthostatic acrocyanosis, spastic tetraparesis, and dystonia. The life span is usually not more than 2 years. In most cases, the clinical presentation arises before seven months, and neonatal form is observed in 25% of patients (2). The exact pathophysiology of the disease is not yet well understood. *ETHE1* gene mutations lead to dysfunction of a mitochondrial dioxygenase involved in hydrogen sulfide (H₂S) detoxification (3). H₂S toxicity has a significant impact on the brain, endothelium, and intestines, as reported by Bulut et al. (2). Additionally,

methyl malonic acid has been previously shown to impair mitochondrial homeostasis through multiple forms. For instance, it inhibits mitochondrial succinate and malate uptake through the mitochondrial dicarboxylate carrier (3). Furthermore, patients during acute encephalopathy crises accumulate and excrete a high amount of lactic acid, suggesting mitochondrial dysfunction (2,4). Biochemical markers including increased blood lactate levels (normal range: 6-22 mg/dl), mild elevation of short-chain acyl-CoA dehydrogenase, with consequent elevation of ethylmalonate and C4/C5 acylcarnitine esters (13). The elevation of ethylmalonic acid level in the

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Received: 19 October 2020 | **Accepted:** 15 December 2020



urine organic acid analysis is a pathogenic marker of the disease. Generally speaking, in the presence of the earlier mentioned clinical manifestations and biochemical findings, the diagnosis of EE is considerably established and should be followed by identification of Pathogenic Biallelic variants in *ETHE1* on molecular genetic testing.

The brain image findings described as symmetric patchy T2-weighted signals in the basal ganglia, periventricular white matter and dentate nuclei, brain stem, and cerebellar white matter are helpful to tailor the diagnosis as well. There is no curative therapy, and the current treatment approach is placing the patient on dual therapy with oral metronidazole and N-acetylcysteine. It has been proposed that the metronidazole decreases exogenous H₂S production by intestinal flora, and N-acetylcysteine replenishes intramitochondrial glutathione stores to neutralize the oxidative injury from H₂S accumulation (4,12). It is also recommended that the patient should avoid nonsteroidal anti-inflammatory drugs given the increased bleeding risk. Liver transplantation was attempted in an infant with EE by Dionisi-Vic et al. (4). Eight months of follow-up after liver transplantation demonstrate an improvement in neurological manifestation with remarkable achievements in psychomotor development and dramatic reversion and normalization of biochemical markers. Here, we describe the phenotype and genotype of patients with EE seen in our center (Supplementary Table 1).

Case Presentation

Family 1

The proband was 14 months old, with a history of global developmental delay more pronounced in the

motor domain; he could not sit yet at the evaluation time, and he did not have any single word. Physical examination revealed microcephaly with a head circumference of below third centile, axial hypotonia, and peripheral hypertonia. Brain manganese resonant (MRI) demonstrates Leukodystrophy findings with the involvement of basal ganglia. Afterward, he was assessed at the metabolic clinic and identified a petechial rash in the extremities and failure to thrive. Family history was significant for one older sibling who passed away at the age of 1 year with a history of fever and septic shock. His Biochemical investigations were substantial for lactic acidosis and mild elevation in the liver enzymes with ALT of 110 IU/l (0-40) and AST of 80 IU/l (0-34). In the constellation of clinical phenotype and the biochemical markers, the diagnosis of EE was suspected; therefore, urine organic acid analysis was performed and showed the high peak of ethylmalonic malonic acid. Hence, full gene sequencing of the *ETHE1* gene was performed and identified to harbor a homozygous pathogenic variant c.487C >T (p.arg163trp) in exon 4. At the time of the diagnosis, his mother had a newborn, and based on the family history, he was evaluated at the age of 14 days. He was asymptomatic. However, the laboratory investigations revealed mild metabolic acidosis with lactate of 3 mmol/l (0.5-2.2 mmol/l) and normal liver enzymes. The target genetic test confirms the diagnosis of EE in him. Both brothers were started on a combination of oral metronidazole and N-Acetylcysteine immediately after diagnosis. There was a mild improvement noticed in gastrointestinal manifestation. However, it was not effective in preventing the disease's progression, particularly neurological manifestations. Both kids passed away at the age of around 2 years of living with a severe metabolic crisis.

Supplemental Table 1. Summary of phenotype and genotype of the patients.

Current age (year)	Age of onset	Neurological Symptoms	Diarrhea	Rash	Lactate (0.5-2.2 mmol/l)	FTT	Urine organic acid	Molecular	Quality of Life	Family history
5	14 days	Hypotonia, GDD	+	+	NA	+	Elevated ethylmalonic acid	c.487C > T (p.Arg163Trp)	Died	+
3	3 months	Hypotonia, GDD, microcephaly, seizure	-	+	3.4	+	Elevated ethylmalonic acid	c.487C > T (p.Arg163Trp)	Died	+
1.5	2 months	Hypotonia, GDD	+	+	4	+	Elevated ethylmalonic acid, isovalerylglycine, isobutyrylglycine	c.487C > T (p.Arg163Trp)	Died	+
2	3 months	GDD, hypotonia, seizure	+	+	3-5	+	NA	c.487C > T (p.Arg163Trp)	Died	-

GDD, Global developmental delay; FTT, Failure to thrive; M, Month; D, Day; NA, Not available; N, normal; (+), Positive, (-), Negative.

Family 2

This is the sixth child of the first cousin's parents. She presented to medical attention with a history of diarrhea since the age of 1 month. She was evaluated extensively by a general pediatrician and labeled to have cow milk protein allergy and had been placed on extensively hydrolyzed formula. However, no improvement had been noticed. Over time, the child required multiple admission with lactic acidosis episodes, which was responding well to hydration for 48 hours. Regarding her development, she started to sit without support at the age of 8 months, crawl at the age of 1 year, and walk at 17 months. She has a total of five words, and there was no concern regarding her communication skills.

Family history is significant for two siblings who passed away at the age of 1 year. The second one was at the period of 7 months with a history of diarrhea and developmental delay. She was seen at the clinic at the age of 2 years and has global developmental and failure to thrive. On examination, her weight and height were below third centile for her age and sex. There was a petechial rash in the extremities and neck. Also, she had mild hypotonia. The biochemical investigation showed evidence of acidosis with lactate of 4 mmol/l (0.5-2.2 mmol/l). There was mildly derange in liver enzymes with ALT 104 IU/l (0-40) and AST of 78 IU/l (0-34). The urine organic acids analysis showed a large peak of ethylmalonic acid. Acylcarnitine profile and plasma amino acids were both normal. Based on the clinical phenotype and biochemical markers, we proceeded with target genetic testing for the same variant identified in our previous patient and confirmed the diagnosis of EE. A homozygous pathogenic variant of c.487C > T (p.Arg163Trp) in exon 4 of *ETHE1* was identified. The child was started in an oral combination of N-Acetylcysteine and Metronidazole, which showed mild improvement in symptoms, but there was no prognosis improvement.

Family 3

This is an 18th month's old girl born to consanguineous marriage. The prenatal history was unremarkable. She was born term with a good Apgar score. At the age of 2 months, she started to have diarrhea and required multiple hydration hospitalizations due to severe metabolic acidosis. Later on, she was identified to have a failure to thrive, low level of activity compared to her peers. Furthermore, she started to show up some neurological symptoms, including central hypotonia, not gaining milestones as expected for her age, and generalized tonic clonic seizures. Family history was noncontributory. Physical examination revealed a weight of less than third centile for her age and sex, petechial rash evolving the chest and upper limb (Figures 1). There was significant axial hypotonia, peripheral hypertonia with hyperreflexia. Biochemical investigation demonstrates lactic acidosis with a lactate level of 5 mmol/l (0.5-2.2 mmol/l). There is a mild elevation of the liver function enzymes ALT of 243 IU/l (0-40), AST of 244 IU/l (0-34) with the normal synthetic function. EE was the working diagnosis. We proceeded with target molecular testing for EE, which

revealed that she harbors a homozygous pathogenic variant c.487C > T (p.Arg163Trp) in exon 4 of the *ETHE1* gene. She had been on an oral combination of N-acetylcysteine and metronidazole, which shows some improvement in GI symptoms, but there was no improvement in neurological manifestation. Despite excellent adherence to the medication child continued to neurological deterioration and passed away with aspiration pneumonia and severe refractory lactic acidosis.

Discussion

EE is a devastating early-onset mitochondrial disease. The *ETHE1* encodes a ubiquitous mitochondrial sulfur dioxygenase involved in the detoxification of H₂S, which is produced in tissues by the catabolism of sulfureted amino acids and, in the large intestine, by anaerobic bacteria (5, 7,6). Most patients who are identified with EE are Mediterranean and Arabs in origin (8). We report the phenotypic and genotypic spectrum of four patients with EE from a single metabolic, genetic center in Oman. All of our patients follow the classical natural history of the disease with variability in the severity of the neurological manifestation. The constellation made the clinical diagnosis of clinical phenotype and laboratory tests. Importantly, urine organic acid analysis, precisely the elevation of Ethylmalonic acid, and hyperlactic acidemia are constant features present in our patients. On the other hand, the Acylcarnitine profile was completely normal in all patients. Given clinical phenotype and the biochemical markers, the Full gene sequencing was pre-formed for the first case identified to have NM_014297.5(*ETHE1*):c.487C > T(p.Arg163Trp) in a homozygous state. The c.487C > T (p.Arg163Trp)



Figure 1. Petechial rash seen in present patient. There is no bleeding diathesis reported.

variant involves the alteration of a conserved nucleotide located at the Metallo-beta-lactamase domain (INterPro). 4/4 in silico tools predict a damaging outcome for this variant. This variant was found in 6/278272 control chromosomes at a frequency of 0.0000216, which does not exceed the estimated maximal expected allele frequency of a pathogenic *ETHE1* variant (0.0013229). It has been reported in multiple affected individuals in the homozygous state (10,11), and a functional study showed the variant with <10% specific activity compared to wild-type. Variants affecting the same codon, such as R163Q, have also been reported to affect individuals, supporting this codon's functional importance (12-13). Taken together, this variant is classified as pathogenic according to ACMG classification. Up until now, generally speaking, there are only 37 variants of *ETHE1* have been reported as disease-causing in EE patients (9). Interestingly, we identify a founder mutation associated with EE in our region. All four patients harbor the same variant, c.487C > T (p.Arg163Trp), and follow up studies for the parents confirmed that they are heterozygous. The variants are considerably severe as all our patients have a poor prognosis and passed away before 4 years.

Further studies are also needed to determine whether this relatively common mutation in the Omani population and if also associated with familial clustering of EE, as well as to verify the clinical value of genetic screening for this mutation.

Conclusion

A c.487C > T (p.Arg163Trp) in exon 4 of *ETHE* gene is the only reported variant associated with EE in Omani patients. Indeed, in the context of a relatively high number and frequency of genetic diseases in the country and the limited resources, screening for these founder mutations should provide a rapid and cost-effective tool for molecular diagnosis of EE in the future. Additionally, these findings should design appropriate measures for carrier screening measures at the normal or regional level.

Funding

None.

Declaration of conflicting interests

The authors of this article have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

Ethical approval

Ethical approval is not required at our institution to publish an anonymous case report.

Consent to participate

An informed consent was obtained from the parents.

Study funding

No targeted funding reported.

Disclosure

The authors declare that they have no conflict of interest.

Ethics statement

The family provided informed consent for inclusion in this report. Approval from a Research Ethics Board is not required at our institution for publication of a case report.

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