Ethylmalonic Encephalopathy

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Summary

Ethylmalonic encephalopathy is a severe mitochondrial disease of early infancy clinically characterised by a combination of developmental delay, progressive pyramidal signs and vascular lesions including petechial purpura, orthostatic acrocyanosis and chronic hemorrhagic diarrhoea. Biochemical hallmarks of the disease are persistently high levels of lactate, and C4–C5-acylcarnitines in blood, markedly elevated urinary excretion of methylsuccinic and ethylmalonic (EMA) acids and defective cytochrome c oxidase (COX) in the muscles. The corresponding gene was named *ETHE1* and mutation analysis including: nonsense and missense mutations, frameshift and deletion of single exons or of the entire gene have been reported. The prognosis of the disease is poor but combined treatment with metronidazole, *N*-acetylcysteine and coenzyme Q10 resulted in disappearance of diarrhoea, petechial showers and acrocyanosis. Ethylmalonic encephalopathy, first described by Burlina et al. 1991, is a severe mitochondrial disease due to mutations in the *ETHE1* gene (MIM≠608451). Clinically it is characterised by a combination of symptoms including developmental delay, progressive pyramidal signs, vascular lesions including petechial purpura, orthostatic acrocyanosis and chronic hemorrhagic diarrhoea. The clinical condition is quite homogeneous but a broad clinical spectrum of severity has been described. The prognosis is poor with half of the patients dying within the first 2 years of life from metabolic decompensation. Brain MRI reveals symmetric patchy signals in basal ganglia, periventricular white matter and dentate nuclei, along with brain and spinal cord malformations. Biochemical hallmarks of the disease are: persistently high levels of lactate, C4–C5-acylcarnitines in blood, markedly elevated urinary excretion of methylsuccinic and ethylmalonic acids and defective COX in the muscles and brain but not in cultured skin fibroblasts. The pathogenic mechanisms underlying the clinical and biochemical abnormalities are the main consequence of ETHE1 impairment. The main consequence of ETHE1 loss is the accumulation of hydrogen sulphide (H_2S), a product of intestinal anaerobes and,

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in trace amount, tissues. Increased concentration of sulphide in tissues (i.e. colonic mucosae, muscle and brain) causes rapid inhibition of COX activity and long-term degradation of COX subunits. In addition, H_2S blocks short-chain fatty acid oxidation by inhibiting the activity of short-chain acyl-CoA dehydrogenase (and possibly other beta-oxidation enzymes as well), which explains the accumulation of EMA. Furthermore, H_2S has vasoactive and vasotoxic effects, which in turn explain the vascular lesions in the skin and, possibly, other organs. Combined treatment with metronidazole, *N*-acetylcysteine and coenzyme Q10 resulted in some neurological improvement, disappearance of diarrhoea, petechial showers and acrocyanosis. Further opportunities for therapy will include transplantation of hemopoietic stem cells and adeno-associated virus mediated gene therapy.

9.1 Introduction

Ethylmalonic encephalopathy (EE) described in this chapter is a very rare mitochondrial disorder caused by mutations in the ETHE1 gene localised to chromosome 19q13. Biochemically, EE is characterised by the unusual combination of severe deficiency of COX, the terminal component of the mitochondrial respiratory chain, in the muscles and brain, leading to high levels of lactate in blood and accumulation of ethylmalonate (ethylmalonic acid, EMA) in urines. EMA, a dicarboxylic organic acid produced by the carboxylation of butyrate, is a hallmark of enzymatic defects of β-oxidation of fatty acids and branched-chain amino acids, for instance defects of the short-chain and branched-chain acylCoA dehydrogenase (SCAD, BCAD). Like in SCAD and BCAD deficiency, accumulation of C4- and C5-acylcarnitines has been documented in blood of EE patients.

The corresponding gene was named *ETHE1* and mutation analysis including nonsense and missense mutations, frameshift and deletion of single exons or of the entire gene have been reported. To date, a total of 70 mutant patients have been identified in our laboratory (Massimo Zeviani unpublished).

The ETHE1 protein, a 30 kDa polypeptide located in the mitochondrial matrix, functions in vivo as a homodimeric, Fe-containing sulphur dioxygenase (SDO) activity, involved in the oxidative detoxification of (harmful) sulphide to (inert) sulphate.

Impaired activity of ETHE1-SDO leads to the accumulation of H_2S in critical tissues, including colonic mucosa, liver, muscle and brain, up to concentrations that inhibit SCAD and COX activities, therefore accounting for EMA aciduria and high levels of C4- and C5-acylcarnitines, EMA and lactate, in plasma. The pathway outlined in Fig. 9.1 explains these findings.

In addition to H_2S, which is a highly unstable, difficult-to-measure gas, its stable derivative thiosulphate is also present at very high concentrations in tissues and body fluids of *Ethe1*$^{-/-}$ mice and EE patients and can well be considered a specific biomarker of the disease.

Besides COX and SCAD deficiency, other symptoms of EE are explained by accumulation of H_2S, including damage of endothelial cells and vasodilation, which account for the petechiae and the acrocyanosis. Since a substantial amount of H_2S derives from the anaerobic bacterial flora residing in the intestinal lumen, COX activity is markedly reduced in the luminal colonocytes of *Ethe1*$^{-/-}$ mice, whereas it is normal in cryptal colonocytes that are relatively secluded from the luminal surface. This difference is likely to reflect the different exposure of the two cell populations to the inhibitory action of exogeneous H_2S. Excessive production and absorption of H_2S, as well as reduced detoxification by colonocytes, is regarded to play an important role in the mucosal damage of ulcerative colitis. A similar mechanism can well account for the severe chronic diarrhoea afflicting EE patients.

9.2 Nomenclature

No.	Disorder	Alternative name	Abbreviation	Gene symbol	Chromosomal localisation	Affected protein	OMIM no.	Subtype
9.1	Ethylmalonic encephalopathy	ETHE1 deficiency	ETHE1	–	19p13.32	Mitochondrial matrix protein	602473	Autosomal recessive

9.3 Metabolic Pathway

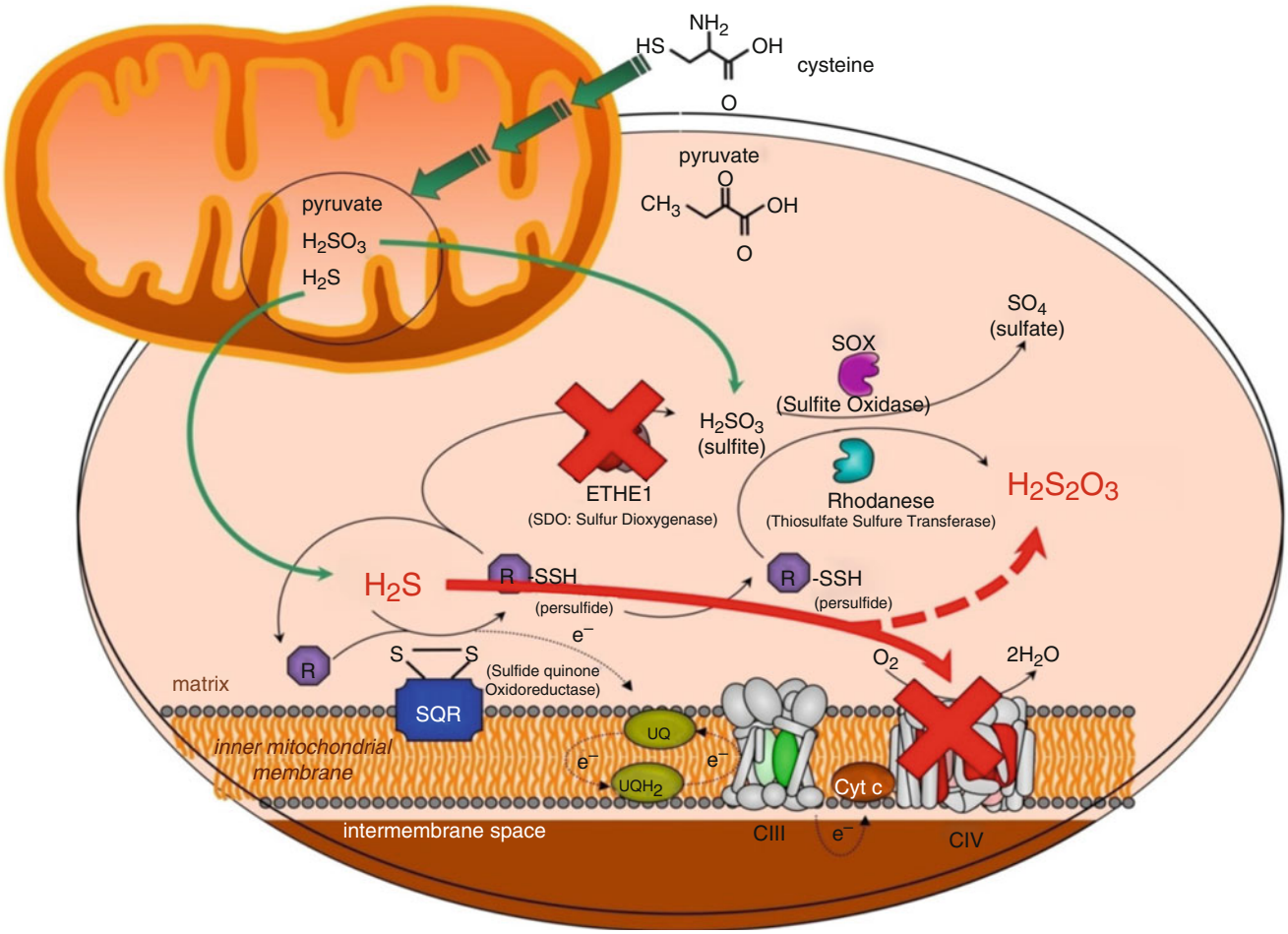

Fig. 9.1 Metabolic pathway of ethylmalonic encephalopathy. From different sources (*green arrow*), through desulphuration and deamination into pyruvate, H_2S (*red arrow*) is initially oxidised, and the sulphur atom is fixed by sulphide–CoQ reductase (*SQR*) to form a persulphide, which is then converted by the sulphur dioxygenase (SDO) activity of Ethe1 into sulphite (SO_3^{2-}). Sulphite is further oxidised to sulphate (SO_4^{2-}) by sulphite oxidase (*SOX*) or exploited as a sulphur acceptor to form thiosulphate ($S_2O_3^{2-}$) by rhodanese. The electrons extracted by SQR are transferred to the mitochondrial respiratory chain via coenzyme Q (Q)

9.4 Signs and Symptoms

Table 9.1 Ethylmalonic encephalopathy

System	Symptom	Neonatal	Infancy	Childhood	Adolescence	Adulthood
Cardiovascular	**Orthostatic acrocyanosis**		+++	+++		
CNS	Hypotonia, axial		++	++		
	Retardation, psychomotor		+++	+++		
	Seizures		++	++		
	Spastic tetraplegia		+++	+++		
	Leigh-like syndrome		+++	+++		
Dermatological	**Petechiae**		+++	+++		
Digestive	Failure to thrive		+++	+++		
	Hemorrhagic diarrhoea, chronic		+++	+++		
Routine laboratory	Hematuria		+	+		
	Lactate (P)		↑↑	↑↑		
	Lactic acidosis		+++	++		
Special laboratory	2-Methylbutyrylglycine (U)		↑↑	↑↑		
	C4 butyryl-carnitine (P, B)	↑↑	↑↑	↑↑		
	C4 isobutyryl-carnitine (P, B)		↑	↑		
	C5 2-methylbutyryl-carnitine (P, B)		↑	↑		
	C5 isovaleryl-carnitine (P, B)		↑	↑		
	C5-DC glutaryl-carnitine (P, B)	↑↑	++	++	++	++
	Ethylmalonic acid (U)	↑↑	↑↑↑	↑↑↑		
	Isovalerylglycine (U)		+	+		
	Methylsuccinic acid (U)		↑↑	↑↑		
	Thiosulphate (U)		+++	+++		

Ethylmalonic encephalopathy is characterised by psychomotor regression and hypotonia, later evolving into spastic tetraparesis, dystonia and eventually global neurologic failure. The encephalopathy is typically accompanied by chronic diarrhoea and failure to thrive.

A clinical trait characteristic of ethylmalonic encephalopathy is the presence of orthostatic acrocyanosis and petechial purpura (Fig. 9.2). This phenomenon has been attributed to the vasoactive and vasotoxic effects of H_2S. Increasing evidence supports an important role for H_2S in the regulation of vascular function, as an endothelium-derived relaxing factor and a regulator of endothelial and smooth-muscle cell growth and apoptosis. However, the vasotoxic effects of H_2S in vivo have not been clearly demonstrated.

Endothelial damage and loss was observed also in the gastric antrum and colonic mucosa and submucosa, associated with microhaemorrhages. Importantly, damage of small vessels in critical organs, notably brain and colon, supports a common pathogenic mechanism in *Ethe1*- patients, centred on the accumulation of hydrogen sulphide to toxic levels.

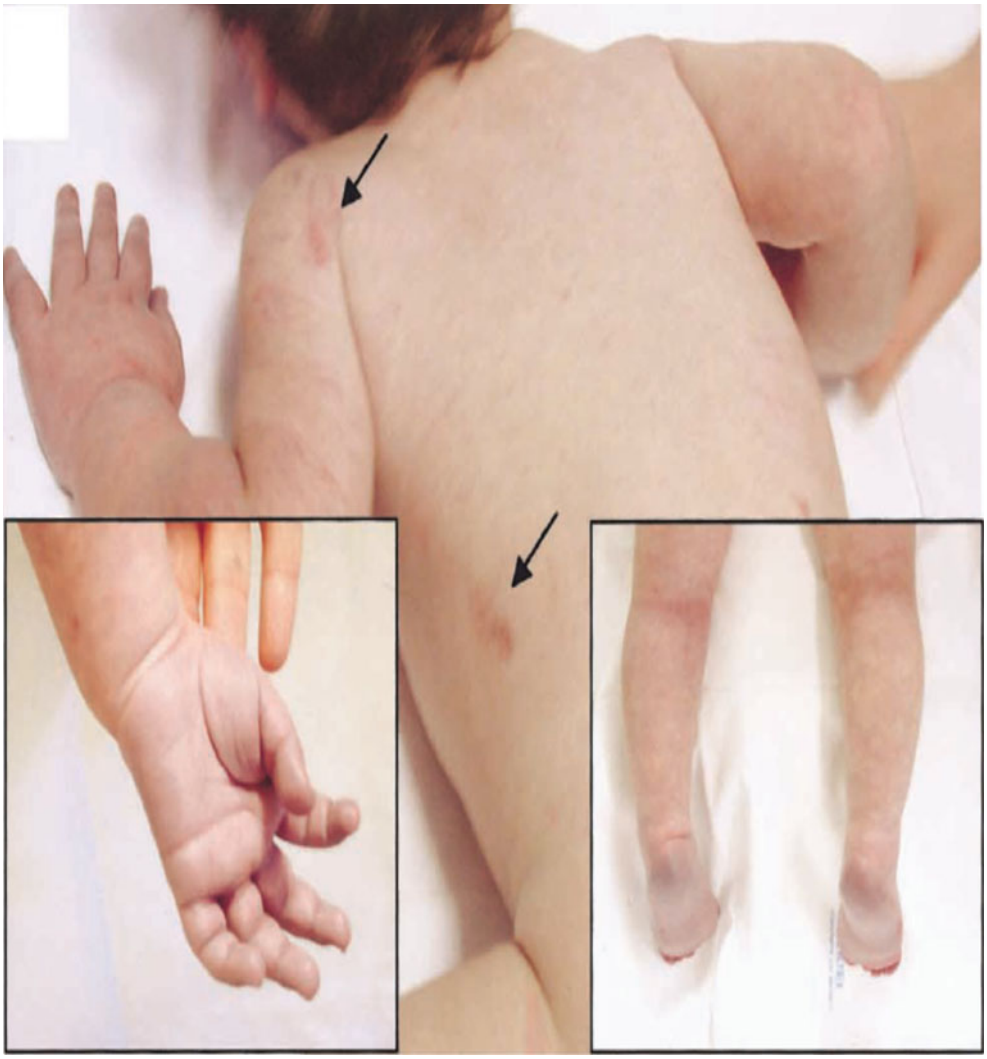

Fig. 9.2 The clinical hallmarks of the ethylmalonic encephalopathy: the widespread lesions of the small blood vessels causing showers of petechiae, easy bruising and orthostatic acrocyanosis (*black arrows*)

Cerebral abnormalities manifest early after birth, with hypotonia, delayed development and, later, spasticity. Generalised seizures are associated with abnormal focal discharges on the electroencephalogram. Neurologic deterioration accelerates following intercurrent infectious illness, and most patients die in the early years of life.

The neuroradiological pattern in EE patients is nonspecific with symmetrical necrotic lesions characterised by high signals on T_2-weighted images in the deep grey matter structures (see Fig. 54.11). These hyperdensities were deemed to be the result of infarcts related to the toxicity of accumulating organic acids (like EMA) or the consequence of vascular changes seen in this disorder. Brain damage was observed in nonvascular areas, such as striatum and brainstem, and underlined the disappearance of these lesions over time, possibly due to brain maturation. Recently, analysis of a brain from an EE patient showed widespread luminal microthrombi, acute microhaemorrhages and focal perivascular haemosiderin-laden macrophages, the latter being consistent with previous bleedings. As a consequence, the brain showed features of both acute and chronic ischaemic damage, consistent with abnormal signal intensity lesions, on repeated MRI. Brain vascular lesions seem to be a specific neuropathological feature of EE due to Ethe1 mutations, as compared to other forms of ethylmalonic aciduria and to disorders caused by primary respiratory chain defects such as Leigh's disease.

9.5 Normal and Pathological Values

Metabolite	Normal	Pathological values
C4-acylcarnitine (B)	0.07–0.50 µmol/l	>3.5 µmol/l
C5-acylcarnitine (B)	0.03–0.50 µmol/l	>1.0 µmol/l
Ethylmalonic (U)	<10 µmol/mmol creat	>60 µmol/mmol creat
Isovalerylglycine (U)	<0.9 µmol/mmol creat	>2 µmol/mmol creat
2-Methylsuccinic (U)	<3 µmol/mmol creat	>10 µmol/mmol creat
2-Methylbutyrylglycine (U)	≤0.1 µmol/mmol creat	>2 µmol/mmol creat
Thiosulphate (U)	100–560 µmol/mg creat	>1,500 µmol/mg creat

9.6 Diagnosis

The main biochemical features of ethylmalonic encephalopathy are increased urinary ethylmalonic and methylsuccinic acids associated with abnormal excretion of C_4 and C_5 (*n*-butyryl-, isobutyryl-, isovaleryl- and 2-methylbutyryl-) acylglycines and acylcarnitines, as well as severe lactic acidosis. Initial laboratory studies in the investigation of ethylmalonic aciduria should include blood glucose, lactate, ammonia, electrolytes and blood gases, a complete blood count, a blood acylcarnitine profile and quantitative urine organic acid analysis by GC-MS. Secondary COX-deficiency in the muscles has been described in several patients. Mutation analysis of the *ETHE1* gene can now provide the definitive diagnosis, including prenatal diagnosis.

Ethylmalonic encephalopathy should be included in the differential diagnosis of persistent ethylmalonic aciduria, which also includes short-chain acyl-CoA dehydrogenase deficiency, defects of the mitochondrial electron-transfer flavoprotein pathway or glutaric aciduria type II and some forms of respiratory chain deficiency.

9.7 Specimen Collection

Test	Specimen	Storage
C4-acylcarnitine	Blood spot, plasma	Keep frozen (−20°)
C5-acylcarnitine	Blood spot, plasma	Keep frozen (−20°)
Ethylmalonic	Urine	Keep frozen (−20°)
Isovalerylglycine	Urine	Keep frozen (−20°)
2-Methylsuccinic	Urine	Keep frozen (−20°)
2-Methylbutyrylglycine	Urine	Keep frozen (−20°)
Thiosulphate	Urine	Keep frozen (−20°)

9.8 Treatment

The prognosis of ethylmalonic encephalopathy is poor, being usually lethal in early childhood. No effective treatment of ethylmalonic encephalopathy is known. Riboflavin, carnitine, ascorbic acid, vitamin E and glycine supplementations all have been tried without benefit, although individual patients were reported to show a slight improvement in motor function, cognitive behaviour and chronic mucoid diarrhoea after treatment with riboflavin and/or coenzyme Q10.

A diet low in branched-chain or sulphur-containing (methionine) amino acids, failed to show any benefit and may in fact be harmful.

Combined exposure to metronidazole, a nitroimidazole anti-infective compound that can buffer the excess of free H_2S, and *N*-acetylcysteine that acts as one of the physiological acceptors of the sulphur atom of H_2S operated by sulphide–CoQ reductase, has been effective in improving the main symptoms in a pilot study on EE patients, including marked attenuation or disappearance of the vascular lesions and diarrhoea, as well as amelioration of some neurological abnormalities.

Recently further opportunities for therapy have been proposed. For instance adeno-associated virus mediated gene targeting could be exploited to express recombinant Ethe1 in the liver, the filter organ that receives blood from the gastrointestinal tract, the major source of exogenous H_2S. A complementary strategy, based on bone marrow transplantation could determine the widespread diffusion of an Ethe1-proficent tissue to promoting the clearance of toxic H_2S from circulating body fluids, thus reducing the exposure of critical organs such as brain and skeletal muscle.

Disorder	Medication	Dosage (mg/kg/day)	Route and frequency
ETHE1 def.	Riboflavin	3–20	Os–2
	Ubiquinone (CoQ10)	5	Os–2
	Metronidazole	25–50	Os–1
	N-acetylcysteine	50–100	Os–2

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