Mitochondria and neonatal epileptic encephalopathies with suppression burst

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Abstract The mitochondrion is a key cellular structure involved in many metabolic functions such as ATP synthesis by oxidative phosphorylation, tricarboxylic acid cycle or fatty acid oxidation. These pathways are fundamental for biological processes such as cell proliferation or death. In the central nervous system, mitochondria dysfunctions have been involved in many neurological diseases and age-related neurodegenerative disorders, including epilepsy, Alzheimer's and Parkinson's diseases. Mitochondrial diseases are frequently caused by a disruption of the respiratory chain. Nevertheless, other mitochondrial functions, including organellar dynamics or metabolite transport, could also be involved in such pathologies. Here we described mitochondrial dysfunctions in a very severe, intractable and relatively rare neonatal epileptic encephalopathy, the Ohtahara syndrome. This condition is characterized by neonatal onset of seizures, interictal electroencephalogram with suppression burst pattern and a very poor outcome with very severe psychomotor retardation or death. The etiology of this disease remains elusive but seems to be very heterogeneous including brain malformations, metabolic errors, transcription factor and synaptic vesicle release defects. In this review, we discuss first the Ohtahara syndrome caused by mitochondrial respiratory chain damages, suggesting that these defects could be more common than previously thought. Then, we will adress the importance of the mitochondrial glutamate carrier SLC25A22 in these pathologies, since mutations of this gene were described in two distinct families. These findings suggest that glutamate metabolism should also be considered as an important cause of the Ohtahara syndrome.

Keywords Ohtahara syndrome · Mitochondria · SLC25A22 · Glutamate carrier

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Introduction

Mitochondria are small cytoplasmic organelles where several metabolic pathways occur including the Krebs and urea cycles, and the β-oxidation of fatty acids. More importantly, mitochondria are essential for cellular energy generation synthesizing adenosine triphosphate (ATP) by oxidative phosphorylation through the mitochondrial respiratory chain, which is composed of five multimeric complexes and two electron carriers: Coenzyme Q and cytochrome c (Fig. 1). Mitochondrial defects are of critical importance in organs requiring a high energy production such as muscles, heart, liver and brain (DiMauro and Schon 2008). Not surprisingly, these organelles are known to be involved in a variety of diseases (http://www.mitomap.org) including cardio-myopathies, cancers, optic neuropathies (Leber hereditary optic neuropathy) and neurodegenerative diseases (Alzheimer's and Parkinson's diseases). We could also observe epileptic phenotypes associated with mitochondrial diseases such as MERRF (Myoclonus Epilepsy with Ragged Red Fibers), MELAS (Mitochondrial Encephalopathy with Lactic Acidosis and Stroke-like episodes) or

Alpers syndrome (for review, see DiMauro and Schon 2008). In addition, mitochondria deficiencies have been recently described in neonatal epileptic encephalopathies with suppression bursts (NEESB) (Williams et al. 1998; Molinari et al. 2005; Castro-Gago et al. 2009; Molinari et al. 2009; Seo et al. 2010).

NEESB is a rare condition characterized by the onset of seizures in the first months of life with an interictal "suppression burst" (SB) electroencephalogram (EEG) pattern. This specific pattern is described as generalized and multifocal, high-voltage, spikes and sharp wave complexes alternating with periods of suppression of the electrical activity (Vigevano and Bartuli 2002). In 1976, Ohtahara and co-workers described early infantile epileptic encephalopathy (EIEE) (Ohtahara et al. 1976) and proposed that this syndrome represented one of three so-called "age-dependent epilepsy encephalopathy syndromes" which also include West and Lennox-Gastaut syndromes (Ohtahara et al. 1977, Yamatogi and Ohtahara 1981). The characteristics of the EIEE syndrome are: onset in early infancy (neonatal period through the first few months of life), tonic spasms (brief tonic seizures) as the predominant seizure type, SB EEG background, medically intractable seizures, severe psychomotor retardation, poor prognosis, and an evolution to West syndrome and then to Lennox-Gastaut syndrome (Ohtahara et al. 1992). Treatments with hormonal therapy (typically ACTH) or antiepileptic drugs (AED) are less effective (Ohtahara and Yamatogi 2003) and the outcome of this condition is poor with the majority of patients either passing away within the first few years of life or surviving in a vegetative state.

A number of etiological factors have been associated to EIEE syndrome comprising brain malformations, Aicardi syndrome, porencephaly, cerebral atrophy or olivary-dendate dysplasia (for review, see Ohtahara and Yamatogi 2003). Mutations in *ARX* and *Munc18-1* genes were also reported in patients with EIEE (Kato et al. 2007; Saitsu et al. 2008; Absoud et al. 2010; Fullston et al. 2010; Kato et al. 2010). And recently, mutations in the gene *SLC25A22*, a mitochondrial glutamate carrier, have been associated with NEESB in two distinct families (Molinari et al. 2005; Molinari et al. 2009). Even through the fact that the Ohtahara syndrome is a well described disease, its pathogenesis seems to be heterogeneous involving transcription factor, synaptic vesicle release and also mitochondrial impairments.

In this review, we will focus on mitochondrial defects, first on complexes deficiencies leading to a respiratory chain dysfunction and resulting in EIEE syndrome. Then, we will discuss the two children with EIEE syndrome caused by *SLC25A22* mutations resulting in a non functional mitochondrial glutamate carrier.

Deficiency of mitochondrial complexes and EIEE

Complex IV or cytochrome c oxidase deficiency

The first case of EIEE described with a specific inborn metabolic disorder was reported in 1998 by Williams and collaborators. The patient was the first child of non consanguineous Asian parents and was born prematurely. His first seizures began at 3 h of age and were difficult to control. Satisfactory treatment with sodium valproate was achieved after 48 days, but seizures recurred after several months of respite. At the age of 2, this child was hypotonic with a severe developmental delay. An EEG performed at the age of 3 months was typical of a SB pattern. Brain MRI performed at 11 months showed a cerebral atrophy. Eye examination was normal but authors did not precise if they further investigated with an electroretinogram (ERG) or visual evoked potential measurements. Metabolic workup results were in normal range but a mitochondrial preparation from a muscle biopsy showed a significant cytochrome c oxidase activity reduction, i.e. respiratory chain complex IV (Fig. 1). The most common mitochondrial mutations were excluded and the complex IV subunit affected remains unknown.

Complex I or NADH coenzyme Q reductase deficiency

Recently, two cases of EIEE syndrome were described with a clear respiratory chain complex I deficiency (Castro-Gago et al. 2009; Seo et al. 2010). In 2009, Castro-Gago et al. described a boy who initiated burst of tonic spasms at 20 days of life. EEG traces showed a typical SB pattern and seizures were refractory to all AED treatments. In addition to his seizures, he presented microsomia, microcephaly, profound mental retardation, and generalized hypotonia. Brain MRI was abnormal and showed an asymmetric dilatation, a thin corpus callosum and a cortical atrophy. Analysis of his cerebrospinal fluid (CSF) revealed an increase in lactic acid levels, and a study of the mitochondrial respiratory chain in a muscle biopsy showed a complex I (NADH coenzyme Q reductase) deficiency with the activities of the remaining complexes in the normal range. The most frequent mitochondrial mutations were excluded as well as deletions or depletion of mitochondrial DNA. This child died at 18 months of age due to multiorgan failure.

In 2010, Seo and collaborators reported the case of a girl, first child of healthy non consanguineous parents, who presented her first seizures on the 5th week. At 3 months of age, her EEG pattern showed SB, she was hypotonic but not dysmorphic, and her brain MRI was normal. Intensive metabolic workup, including lactic acid level in CSF, showed no significant changes but a magnetic resonance spectroscopy revealed high lactate peaks in the right basal ganglia and the frontal white matter. Mitochondrial respi-
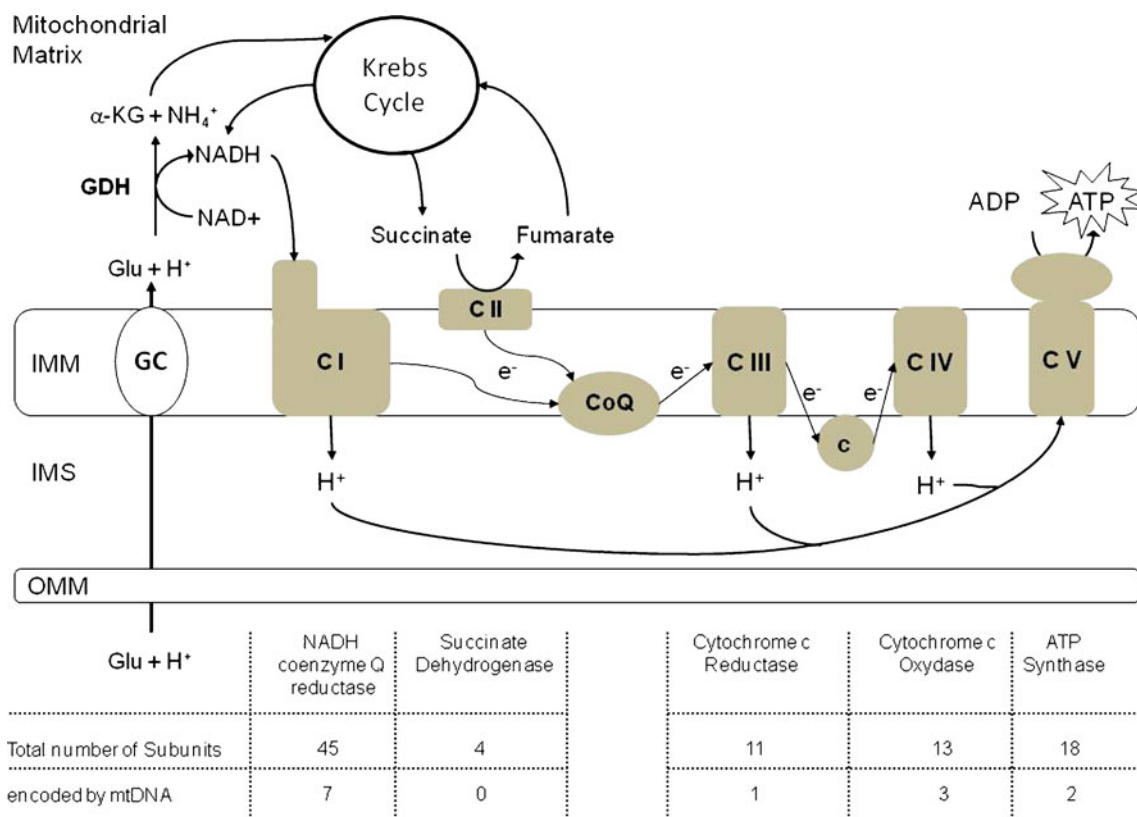
Fig. 1 Mitochondrial Respiratory Chain (MRC). The MRC is composed of 4 multimeric complexes located in the inner mitochondrial membrane (IMM): Complex I (C I or NADH coenzyme Q reductase); Complex II (C II or succinate dehydrogenase); Complex III (C III or cytochrome c reductase); and Complex IV (C IV or cytochrome c oxidase). Complex V (C V), separate from the respiratory chain, is the ATP synthase. The subunit composition of each complex is indicated in the above table. Protons (H^+) are pumped from the mitochondrial matrix to the intermembrane space (IMS) through CI, CIII, and CIV and electrons (e) are transferred between the Complexes I and III via coenzyme Q (CoQ) and between Complex III and IV via Cytochrome c (c). Protons accumulated in the IMS are then used by C V to drive the synthesis of ATP from ADP and phosphate. The glutamate carrier (GC) transports a molecule of glutamate (Glu) with a proton from the cytoplasm to the matrix. There, the glutamate dehydrogenase (GDH) catalyzes conversion of Glu into α-ketoglutarate (α-KG) and ammonium. The α-KG then enters the Krebs cycle. The GDH reaction produces also NADH, the subtrate of C I. (OMM = outer mitochondrial membrane)

ratory chain analysis from a fresh muscle biopsy revealed a significant decrease of complex I activity whereas the other complexes activities were normal. Seizures were refractory to classical AED treatments (adrenocorticotropic hormone, phenobarbital, sodium valproate) but she showed a decrease of 80% of her seizures frequency with ketogenic diet and mitochondrial cocktail supplementation.

The complex I is the largest complex of the mitochondrial respiratory chain and is composed of 45 subunits (7 encoded by mitochondrial DNA, see Fig. 1; Carroll et al. 2006). In these two cases, the complex I subunit(s) involved is/are still unknown.

Mitochondrial glutamate transport impairment and EIEE

Mutations in the *SLC25A22* gene, localized on chromosome 11p15.5 and encoding a mitochondrial glutamate carrier, were identified in two families with children presenting NEESB (Molinari et al. 2005; Molinari et al. 2009).

The SLC25A22 protein is located in the inner mitochondrial membrane and catalyzes a glutamate/H+ symport into the mitochondria (Fig. 1; Palmieri 2004). The first mutation was identified in children (2 boys and 2 girls) born from first cousin Arab Muslim parents from Jerusalem (Molinari et al. 2005). Genetic and sequencing analysis pointed out a homozygous mutation in exon 8 of the *SLC25A22* gene, changing a highly conserved amino-acid proline into a leucine (p.Pro206Leu). The screening of 30 patients presenting epileptic encephalopathies was performed and a second mutation was identified in a boy born from Algerian first cousin parents (Molinari et al. 2009). The mutation identified changed also a highly conserved amino-acid, a glycine into a tryptophan (p.Gly236Trp). Analysis of the glutamate exchange and transport of these two mutated proteins were tested with an *in vitro* model of reconstituted proteoliposomes (Palmieri et al. 1995). These

experiments showed that both mutated proteins could not catalyze transport or exchange of glutamate (Molinari et al. 2005; Molinari et al. 2009). Interestingly, analysis of the mitochondrial respiratory chain using patient cultured skin fibroblasts showed a normal cell respiration in the first family. However, after permeabilization of cell membranes by digitonin and in the presence of a specific inhibitor of amino-aspartate transferase, the patient cells failed to oxidize glutamate while oxidation of a different substrate, such as succinate, was normal (Molinari et al. 2005). This experiment confirmed that glutamate could not enter into the patient cells due to a transport defect. Moreover, it showed that the mitochondrial respiratory chain is functional which could explain why we never detected any metabolic errors in patients, at least in the tissues tested. This could also be explained by the presence of another mitochondrial glutamate carrier, SLC25A18, localized on chromosome 22q11.2, which catalyzes glutamate/H+ symport into the mitochondria (Fiermonte et al. 2002). This second isoform could compensate the absence of SLC25A22 in the majority of the tissues but not in the brain where SLC25A22 is highly expressed within areas involved in motor coordination and eye movement (Molinari et al. 2005; Molinari et al. 2009).

The clinical analysis of these five patients suggested that mutations on SLC25A22 mutations could be responsible for a recognizable EIEE syndrome. Indeed, all these patients presented the same characteristics: epileptic spasms and focal seizures associated to SB in the first days of life, microcephaly, hypotonia, abnormal ERG recording and lack of psychomotor development. In addition, brain imaging of two children from both families revealed cerebellar hypoplasia, callosal dysmorphia, abnormal gyration of temporo-parietal regions, and abnormal myelination of temporal poles (Molinari et al. 2009).

Conclusion

The neonatal epileptic encephalopathy with suppression burst is a very rare condition whose evolution is often severe since patients either survive in a lethargic state or die. Despite great progress in understanding the molecular basis of epilepsy, the pathogenesis of NEESB still remains unclear.

Several static brain malformations and lesions have been described for the Ohtahara syndrome: hemimegalencephaly, porencephaly, Aicardi syndrome, olivary-dendate dysplasia, agenesis of mamillary bodies, cerebral dysgenesis, and focal cortical dysplasia (reviewed in Ohtahara and Yamatogi 2003). Inborn metabolic disorders involving mitochondria have also been reported in few cases of EIEE, involving complexes I and IV which were described here. We could hypothesize

that deficiencies in cytochrome c oxidase and NADH coenzyme Q reductase might have resulted in abnormal neuronal migration or demyelination by depletion of energy during a critical period of brain development.

Recently, three genes involved in the Ohtahara syndrome without any brain damage were described in the literature. Specific mutations of the ARX (aristaless-related homeobox) gene at Xp22.13 have been recently found in male subjects with EIEE syndrome (Kato et al. 2007; Fullston et al. 2010; Absoud et al. 2010; Kato et al. 2010). ARX is expressed predominantly in the fetal and adult brain, testis, skeletal muscle, and pancreas. And, even if its role is still less understood, Arx is specifically involved in radial and tangential migration of GABAergic neuron progenitors, and early commitment of cholinergic neurons (Kitamura et al. 2002; Colombo et al. 2007; Friocourt et al. 2008). Mutations in the STXBP1 (MUNC18-1) gene at 9q34.1 were described in five patients presenting EIEE characteristics (Saitsu et al. 2008). STXBP1 is a highly conserved neuronal protein that is essential in synaptic vesicle release (Verhage et al. 2000) and which participates also in the regulation of calcium channels (Khanna et al. 2007). Neurotransmitter release, such as glutamate and GABA, is essential in early brain development since these molecules regulate neuronal progenitor cells proliferation (LoTurco et al. 1995) and neuronal migration (Behar et al. 1999). Dysregulation of synaptic vesicle release could lead to abnormal cell migration, miscommunication between neurons resulting in abnormal brain activities and to epileptic phenotypes (Manent et al. 2005). Recently, it has also been shown that cell surface distribution of STXBP1 could be enhanced by the extracellular glutamate concentration (Wan et al. 2010), which is of critical importance in a number of neurodegenerative diseases and epileptic syndromes. We could hypothesize that glutamate metabolism has an important role in the development of EIEE since the third gene involved in such pathologies is SLC25A22, a mitochondrial glutamate carrier. Interestingly, SLC25A22 is more abundant in astrocytes than in neurons and data suggest that glutamate uptake in astrocytes is assumed only by SLC25A22 (Berkich et al. 2007). It is worth remembering that regulation of extracellular glutamate concentration is mainly controlled by astrocytes (Danbolt 2001). Absence of functional SLC25A22 could result in accumulation of glutamate in astrocytes and lead to a dysregulation of extracellular glutamate. This extracellular glutamate could enhance STXBP1 distribution at the neuronal surface, leading to STXBP1 antibody-induced neuronal injuries observed in some pathogenesis as Rasmussen's encephalitis (Yang et al. 2000). Moreover, glutamate could also diffuse and activate extrasynaptic glutamate receptors, resulting in CREB shut-off pathway (Hardingham et al. 2002), neuronal death induction

(Hardingham et al. 2002) and inactivation of the ERK signaling cascade (Ivanov et al. 2006).

In conclusion, the pathogenesis of EIEE is heterogeneous and there are still no effective therapies for these conditions. Better understanding the pathogenesis of these syndromes could help clinicians to improve drug treatments. Specifically, defects in mitochondria, with or without mitochondrial respiratory chain impairment, and in glutamate metabolism should be considered as important causes of these pathologies.

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