A Mutation in the SLC25A12 Gene Reveals its Critical Roles in N-acetylaspartate Formation, Myelination and Brain Function

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The SLC25A12 gene encodes the mitochondrial aspartate–glutamate carrier (denoted as aralar or AGC1), an essential component of the malate–aspartate shuttle that facilitates the exchange between intramitochondrial aspartate and cytosolic glutamate plus a proton (see Figure 11-4 in chapter) (see chapter text and references therein). A mutation in AGC1 has recently been identified in humans, and data from the first patient and from studies in aralar knockout mice link this carrier to neuronal synthesis of N-acetylaspartate (NAA), synthesis of myelin lipids in oligodendroglia in developing brain, and overall brain function (see Box Figure 11.1).

Brain energetics requires a redox shuttle system to regenerate cytoplasmic NAD⁺ for continuation of glycolysis and production of pyruvate as substrate for oxidative metabolism. In brief, the oxidation–reduction (shown in orange in Figure 11-4) and transamination (shown in turquoise in Figure 11-4) reactions of the malate–aspartate shuttle transfer reducing equivalents from cytoplasm into mitochondria for ATP production. Aspartate formed within neuronal mitochondria is also a precursor for synthesis of NAA, and oligodendroglia are known to hydrolyze NAA to provide acetate for synthesis of specific myelin lipids (Burri et al., 1991; Chakraborty et al., 2001). Indeed, a deficiency of NAA-derived acetate may contribute to the pathology of Canavan’s disease (Madhavarao et al., 2005; Moffett et al., 2007; Arun et al., 2010), in which a lack of the aspartoacylase enzyme prevents the hydrolysis of NAA.

The role of the neuronal aspartate–glutamate carrier (aralar, AGC1) in N-acetylaspartate (NAA) formation and trafficking and providing acetyl groups for synthesis of myelin lipids.

Cloning of aralar led to the development of knockout mice, identification of human
mutations, and a better understanding of the essential roles of aspartate formation and release from neuronal mitochondria in brain function. Mice with a complete knockout of aralar (Aralar\(^{-/-}\)) are developmentally delayed and die by postnatal day 22. Aralar\(^{-/-}\) mice have a profound decrease in the concentrations of aspartate and NAA without apparent neuronal loss, and the patient with a mutation in the SLC25A12 gene has low brain NAA levels. Aralar\(^{-/-}\) mice also have hypomyelination and low concentrations of specific myelin lipids, consistent with cerebral hypomyelination in the aralar-deficient child, who also had severe psychomotor developmental delay and seizures. Mitochondria isolated from muscle of the aralar-deficient child had a non-functional carrier and severely impaired respiration with glutamate. Since brain lactate level was not elevated in this child, Wibom et al. (2009) questioned the importance of the malate–aspartate shuttle in brain and raised the possibility of a compensatory shuttle system.

Use of neuronally generated NAA by oligodendrocytes and reduced NAA levels without apparent neuronal loss validate the concept proposed by Clark (1998) that NAA is a marker of neuronal mitochondrial function rather than of neurons per se. This distinction has important implications for interpretation of \(^1\)H-NMR spectra used for clinical diagnosis because a decrease in NAA or ratio of NAA/total creatine is often interpreted as a loss of neurons (Moffett et al., 2007; Clark, 1998; Bates et al., 1996). However, decrements in NAA level can also represent reversible neuronal or mitochondrial dysfunction (Moffett et al., 2007).

Mitochondrial respiration is essential for neurons, and the question of whether there is a compensatory redox shuttle system is important. Two alternatives are the glycerol phosphate shuttle and the aspartate–glutamate carrier, citrin (AGC2). Upregulation of citrin, which can substitute for aralar in AGC1 deficiency, could preserve malate–aspartate shuttle function; residual expression of citrin could explain the regional hypomyelination in the patient. Low
levels of citrin are found in specific neuronal clusters in mouse brain (Contreras et al., 2010); this finding should stimulate studies to evaluate citrin levels in *Aralar*−/− mice and in human brain.

In summary, studies of aspartate–glutamate carrier deficiency highlight the critical multifunctional roles of a single neuronal mitochondrial protein and a single amino acid (aspartate). This carrier is essential for brain energetics, intra- and intercellular metabolite trafficking for biosynthesis of key lipid components of the myelin sheath, neuron-oligodendroglia interactions and overall brain development and function. Reduced aspartate formation and/or efflux from neuronal mitochondria leads to reduced NAA levels, hypomyelination, and severe neurological defects. These findings raise a number of important issues, including the lack of lactate accumulation and possibility of alternative shuttle systems, the origin of the 4-carbon backbone for neuronal aspartate that is used to synthesize and export NAA, and the fate of the aspartate generated by hydrolysis of NAA in oligodendroglia. Thus, detailed analysis of the phenotypes associated with mutations in critical brain proteins can lead to a better understanding of the interactions among seemingly unrelated pathways and among neurons, oligodendroglia and astrocytes.

**References**


Burri et al., 1991 R. Burri, C. Steffen, N. Herschkowitz, N-acetyl-L-aspartate is a major source


