Pump-Opathies: Mutations in Na\(^+\)-K\(^+\)-ATPase Genes Produce Severe Developmental Epileptic Encephalopathies

**ATP1A2- and ATP1A3-Associated Early Profound Epileptic Encephalopathy and Polymicrogyria**

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Constitutional heterozygous mutations of ATP1A2 and ATP1A3, encoding for 2 distinct isoforms of the Na\(^+\)/K\(^+\)-ATPase (NKA) alpha-subunit, have been associated with familial hemiplegic migraine (ATP1A2), alternating hemiplegia of childhood (ATP1A2/A3), rapid-onset dystonia-parkinsonism, cerebellar ataxia-ataxia-progressive ophthalmopathy, and relapsing encephalopathy with cerebellar ataxia (all ATP1A3). A few reports have described single individuals with heterozygous mutations of ATP1A2/A3 associated with severe childhood epilepsies. Early lethal hydrops fetalis, arthrogryposis, microcephaly, and polymicrogyria have been associated with homozygous truncating mutations in ATP1A2. We investigated the genetic causes of developmental and epileptic encephalopathies variably associated with malformations of cortical development in a large cohort and identified 22 patients with de novo or inherited heterozygous ATP1A2/A3 mutations. We characterized clinical, neuroimaging, and neuropathological findings; performed in silico and in vitro assays of the mutations' effects on the NKA-pump function; and studied genotype–phenotype correlations. Twenty-two patients harbored 19 distinct heterozygous mutations of ATP1A2 (6 patients, 5 mutations) and ATP1A3 (16 patients and 14 mutations, including a mosaic individual).

Polymicrogyria occurred in 10 (45%) patients, showing a mainly bilateral perisylvian pattern. Most patients manifested early, often neonatal, onset seizures with a multifocal or migrating pattern. A distinctive, “profound” phenotype, featuring polymicrogyria or progressive brain atrophy and epilepsy, resulted in early lethality in 7 patients (32%). In silico evaluation predicted all mutations to be detrimental. We tested 14 mutations in transfected COS-1 cells and demonstrated impaired NKA-pump activity, consistent with severe loss of function. Genotype–phenotype analysis suggested a link between the most severe phenotypes and lack of COS-1 cell survival, and also revealed a wide continuum of severity distributed across mutations that variably impair NKA-pump activity. We performed neuropathological analysis of the whole brain in 2 individuals with polymicrogyria, respectively, related to a heterozygous ATP1A3 mutation and a homozygous ATP1A2 mutation and found close similarities with findings suggesting a mainly neural pathogenesis, compounded by vascular and leptomeningeal abnormalities. Combining our report with other studies, we estimate that 5% of mutations in ATP1A2 and 12% in ATP1A3 can be associated with the severe and novel phenotypes that we describe here. Notably, a few of these mutations were associated with more than 1 phenotype. These findings assign novel, “profound,” and early lethal phenotypes of developmental and epileptic encephalopathies and polymicrogyria to the phenotypic spectrum associated with heterozygous ATP1A2/A3 mutations and indicate that severely impaired NKA-pump function can disrupt brain morphogenesis.

**Commentary**

To regard the sodium potassium pump (Na\(^+\)/K\(^+\)-ATPase) as the workhorse of neuronal and glial membrane homeostasis is an understatement. While this pump is best known for maintaining the cell’s resting membrane potential, it plays many other critical roles in cell function, including restoration of Na\(^+\) and K\(^+\) transmembrane gradients after neuronal firing, regulation of cell volume, information processing, synaptic plasticity, intrinsic firing, afterhyperpolarization, and even regulation of glucose utilization. As discussed below, some of these functions are becoming apparent with the discovery of human mutations of genes coding for pump components.

Na\(^+\)/K\(^+\)-ATPase is a large molecule and one that is energetically hungry, consuming ~50% of the cell’s energy to pump Na\(^+\) and K\(^+\) against their concentration gradients. The pump exports 3 Na\(^+\) ions out of the cell and imports 2 K\(^+\) ions into the cell for each ATP molecule hydrolyzed. Structurally, Na\(^+\)-K\(^+\)-ATPase
comprises a large subunit (α) and 2 smaller subunits (β, γ). The catalytic α subunit contains ATP and ion-binding sites and has 2 major isoforms in brain (α2 and α3, encoded by \(ATP1A2\) and \(ATP1A3\), respectively); the β subunit targets the α subunit to the membrane, and the γ subunit modulates the affinity of the α subunit for \(K^+\) or \(Na^+\).\(^2\) The expression of \(Na^+\)/\(γ\) membrane, and the \(ATP1A3\) catalytic major isoforms in brain (sources and databases. Mutations span the molecule, including ion-binding sites, phosphorylation sites, and protein-folding domains. On structural analysis, 10 of the 22 children had polymicrogyria on brain MRI scans predominantly over perisylvian cortex (verified in 2 autopsied patients), suggesting disrupted neuronal migration. Brain atrophy, when present, was highly associated with early mortality. Nine of the 10 children with polymicrogyria had mutations in the \(ATP1A3\) isoform. 18 of the 22 children had global developmental delays, most of them in the severe or profound range. In summary, clinical data suggests that one-third of the reported patients had a distinctive syndrome of severe early-onset multifocal epilepsy, often lethal, and polymicrogyria with progressive brain atrophy.

To explore the biochemical and functional consequences of \(Na^+/K^+\)-ATPase mutations, the authors used COS-1 cells (transfection of which produces recombinant proteins) with endogenous \(Na^+/K^+\)-ATPase knocked down. When these cells were transfected with mutant protein, most died, with only 5 mutations having sufficient \(Na^+/K^+\)-ATPase protein to generate sufficient pump function to survive, but even those surviving cells harbored physiological abnormalities. The impaired pump activity was consistent with severe loss of function, with a wide spectrum of severity across mutations affecting pump activity. Since most mutations were unable to support cell viability in culture, the \(Na^+/K^+\)-ATPase pump is considered to be essential for cell survival. Of the mutants that retained some pump transport activity and survived, there were mutations in the ability of both \(Na^+\) and \(K^+\) to bind to their appropriate sites, as well as effects on conformational changes of the molecule and phosphorylation capacity. Therefore, multiple potential pathological disturbances were detected underlying pump dysfunction due to the different mutations, with diverse functional consequences not intimately correlated with the specific mutation. Clearly, many additional patients (and thus mutations) will be necessary to generate a clearer picture of the range and type of pump dysfunction.

This paper expands understanding of the roles of \(Na^+/K^+\)-ATPase and its mutations, from its critical function in metabolic regulation and ion homeostasis, to a more expansive picture involving developmental epileptic encephalopathy and abnormalities in brain morphogenesis and neuronal migration. It remains unclear how \(Na^+/K^+\)-ATPase mutations lead to structural cortical malformations like polymicrogyria, why seizures are so common when pump function is lost, and whether information gained from studies such as this will lead to therapeutic options. As evident in the burgeoning literature on genotype-phenotype correlation in other neurological disorders, the challenges for clinicians and researchers are become more complex (and therefore more interesting!).

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