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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-areflexia-progressive opticatrophy, 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.[1,2] 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^+–K^+ pump isoforms is tissue specific and subject to developmental and pathological changes. In brief, the presence of Na^+ and ATP intracellularly allows binding of Na^+ to Na^+/K^+-ATPase in what is called the E1 conformation. Hydrolysis of ATP phosphorylates E1 and permits extracellular Na^+ release. The resultant E2 conformation binds 2 extracellular K^+ ions, dephosphorylation of which allows intracellular release of K^+ and repeat of the cycle. Thus, there are multiple potential sites of mutation and physiological dysfunction, with widespread mutations already identified.

It has been known for some time that mutations in Na^+/K^+-ATPase are responsible for the autosomal dominant disorders, familial hemiplegic migraine (FHP, *ATP1A2*), and alternating hemiplegia of childhood (AHC, *ATP1A3*). Other rare conditions are also caused by *ATP1A2/3* mutations, including rapid-onset dystonia-parkinsonism and cerebellar ataxia-areflexia-progressive optic atrophy.[3] All of those disorders are episodic in nature, giving rise to the concept that *ATP1A* mutations have paroxysmal manifestations. For example, seizures are described in about half of children with AHC and in up to one-third of those with FHM, but a more severe syndrome has also been described, setting the stage for the current work.[4] Furthermore, it is now apparent that chronic and progressive disorders can also arise from *ATP1A* mutations.[5]

The present article describes a previously underappreciated spectrum of severe developmental epileptic encephalopathies associated with *ATP1A* mutations and expands the underlying spectrum of neurological mechanisms in this range of disorders.[6] The authors report genotype–phenotype correlations in 22 patients with *de novo* or inherited heterozygous *ATP1A2/3* mutations. The 22 patients were identified from referrals for developmental epileptic encephalopathy supplemented by additional patients gleaned from international sources and databases. Mutations span the molecule, including ion-binding sites, phosphorylation sites, and protein-folding domains.

Seizures were almost universal in this cohort, occurring in 21 of the 22 cases. Seizures typically began in the neonatal period or in early childhood and were generally severe in nature. Ten children, 5 of them infants, presented with refractory status epilepticus. Eight of these ten children died. Seizure semiology was multifocal and migrating focal, raising the possibility that some of these children fit into the clinical spectrum of the syndrome known as early infantile multifocal seizures (EIMFS). Eight children had seizure-related apnea episodes, thought to represent central control dysfunction, as brainstem morphology was unremarkable. Overall, the predominance of severe seizures suggests that circuit cell dysfunction is present when the pump is not present or is dysfunctional, adding to the disruption of metabolic regulation seen in Na^+/K^+-ATPase disorders.[7,8]

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 pathophysiological 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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