Published in final edited form as:
Lancet Neurol. 2014 May ; 13(5): 503–514. doi:10.1016/S1474-4422(14)70011-0.

Distinct neurological disorders with ATP1A3 mutations

Erin L. Heinzen, Ph.D.[1,2], **Alexis Arzimanoglou**[3,4], **Allison Brashear, M.D.**[5], **Steven J. Clapcote, Ph.D.**[6], **Fiorella Gurrieri, Ph.D.**[7], **David B. Goldstein, Ph.D.**[1,8], **Sigurður H. Jóhannesson**[9,10], **Mohamad A. Mikati, M.D.**[11,12], **Brian Neville, FRCPCH**[13], **Sophie Nicole, Ph.D.**[14,15,16], **Laurie J. Ozelius, Ph.D.**[17,18], **Hanne Poulsen, Ph.D.**[19,20], **Tsveta Schyns, Ph.D.**[21], **Kathleen J. Sweadner, Ph.D.**[22], **Arn van den Maagdenberg, Ph.D.**[23,24], **Bente Vilsen, D.M.Sc**[25], and **ATP1A3 Working Group**

[1]Center for Human Genome Variation, Duke University, School of Medicine, Durham, NC 27708, USA [2]Department of Medicine, Section of Medical Genetics, Duke University, School of Medicine, Durham, NC 27708, USA [3]Epilepsy, Sleep and Pediatric Neurophysiology Department, HFME, University Hospitals of Lyon (HCL), Lyon, France [4]CRNL, CNRS UMR 5292, INSERM U1028, Lyon, France [5]Department of Neurology, Wake Forest School of Medicine, Winston Salem, NC 27157, USA [6]School of Biomedical Sciences, University of Leeds, Leeds LS2 9JT, UK [7]Istituto di Genetica Medica, Università Cattolica S. Cuore, Rome, Italy [8]Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, North Carolina, USA [9]AHC Federation of Europe (A.H.C.F.E.) - Jórusel 18, 109, Reykjavík, Iceland [10]AHC Association of Iceland, Reykjavik, Iceland [11]Division of Pediatric Neurology, Duke University Medical Center, Durham, North Carolina, USA [12]Department of Neurobiology, Duke University, Durham, North Carolina, USA [13]Institute of Child Health, University College London, London, UK [14]Institut National de la Santé et de la Recherche Médicale, U975, Centre de Recherche de l'Institut du Cerveau et de la Moelle, Paris, France [15]Centre National de la Recherche Scientifique, UMR7225, Paris, France [16]Université Pierre et Marie Curie Paris VI, UMRS975, Paris, France [17]Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA [18]Department of Neurology, Icahn School of Medicine at Mount Sinai, New York, NY, USA [19]Danish Research Institute for Translational Neuroscience, Nordic-EMBL Partnership

Corresponding Author. Erin L. Heinzen, Ph.D., 308 Research Drive, Box 91009, 327C Levine Science Research Building, Durham, NC 27008, (P) 919-684-8684, (F) 919-668-6787, e.heinzen@duke.edu.

Search strategy and selection criteria

References cited in this review were identified through PubMed searches using the search terms "ATP1A3", "alternating hemiplegia of childhood", "rapid-onset dystonia parkinsonism", and "Na, K-ATPase", from December, 1993, until January, 2014. Articles were identified through searches of the reference lists of the articles found with the above cited search terms and of the authors' own files. All references used in this review were published in English, and were assembled based on originality and relevance to the content of this review.

Author contributions

ELH, AB, SJC, DBG, SJ, MM, BN, LJO, HP, TS, KJS, AVDM, BV compiled relevant information from the literature and wrote the manuscript. The entire ATP1A3 Working Group critiqued and edited the manuscript, and provided scientific and clinical guidance regarding the content.

of Molecular Medicine, Department of Molecular Biology and Genetics, Aarhus University, Denmark [20]Centre for Membrane Pumps in Cells and Disease-PUMPKIN, Danish National Research Foundation, Denmark [21]European Network for Research on Alternating Hemiplegia (ENRAH) [22]Neurosurgery, Massachusetts General Hospital, Boston, Massachusetts, USA [23]Department of Human Genetics, Leiden University Medical Centre, P.O. Box 9600, 2300 RC Leiden, The Netherlands [24]Department of Neurology, Leiden University Medical Centre, P.O. Box 9600, 2300 RC Leiden, The Netherlands [25]Department of Biomedicine, Aarhus University, Denmark

Abstract

Genetic research has shown that mutations that modify the protein-coding sequence of *ATP1A3*, the gene encoding the α3 subunit of Na^+/K^+-ATPase, cause both rapid-onset dystonia parkinsonism and alternating hemiplegia of childhood. These discoveries link two clinically distinct neurological diseases to the same gene, however, *ATP1A3* mutations are, with one exception, disease-specific. Although the exact mechanism of how these mutations lead to disease is still unknown, much knowledge has been gained about functional consequences of *ATP1A3* mutations using a range of in vitro and animal model systems, and the role of Na^+/K^+-ATPases in the brain. Researchers and clinicians are attempting to further characterise neurological manifestations associated with mutations in *ATP1A3*, and to build on the existing molecular knowledge to understand how specific mutations can lead to different diseases.

Introduction

The introduction of next-generation sequencing technology, which allows nearly complete assessment of human exomes and genomes, has significantly boosted gene discovery.[1] These gene discoveries have advanced our knowledge of disease-specific pathophysiology, and have enabled genetic connections to be made between diseases. An example is the recent identification of mutations in *ATP1A3*, a gene previously associated with rapid-onset dystonia parkinsonism (RDP), in alternating hemiplegia of childhood (AHC). This genetic connection between diseases offers unique research opportunities to explore how genetics governs specific clinical phenotypes, and to study the underlying pathophysiology.

Here, we review current knowledge of the roles of ATP1A3 in the brain, as well as the phenotypes associated with mutations in the gene. Finally, we discuss additional research needed to further characterise the full phenotypic spectrum associated with mutations in *ATP1A3* and the associated changes in protein function, to establish possible strategies for the development of new treatments for AHC, RDP, and related disorders.

Roles of Na^+/K^+-ATPases

Na, K-ATPases are membrane-bound transporters that harness the energy of hydrolysis of single ATPs to move three sodium ions (Na^+) out of the cell in exchange for two potassium ions (K^+) moving inwards. The resulting ionic gradients are used to establish membrane potentials that are used to generate electrical impulses, and to move neurotransmitters and

calcium ions (Ca^{2+}) across the plasma membrane. Na, K-ATPases are comprised of catalytic α, β, and regulatory γ subunits (Figure 1). The main role of the α subunit is to bind and transport Na^+ and K^+. There are four α subunits all encoded by different genes. The α3 subunit, encoded by *ATP1A3*, is the predominant α subunit expressed in neurons,[2–4] although many neurons also express α1. The α3 subunit differs from α1 in the sense that it has a relatively low affinity for Na^+ and K^+,[5,6] which enables a rapid normalization of ion gradients after intense neuronal firing. Changes in the activity of Na, K-ATPases in neurons (predominantly expressing α3) have been shown to have various physiological consequences. For example, inhibition of Na, K-ATPase in the thalamus converted neuronal bursting responses to single spike discharges.[7] In hippocampus, a reduction of the activity of Na, K-ATPase caused interictal epileptiform bursting activity.[8] Furthermore, localisation of α3 in dendritic spines has been shown to play a part in controlling the size and speed of the small depolarisations caused by fluctuations of intracellular Na^+ that occur during activation of ion gating neurotransmitter receptors. These fluctuations, known as transients, are summated in dendrites and are the basis of synaptic integration.[9–11]

In addition to its primary function in ion transport, subunits of Na+/K+-ATPases have been shown to interact with proteins that assist in localisation of enzymes to the cell membrane, modulate the PI3K, PLCγ, and MAPK signal transduction cascades, and regulate the activity of other transporters and receptors.[12]. The protein-protein interactions specific to theα3 subunit are not fully characterized, but, as an example, the α3 isoform was shown in rat neurons to bind specifically to Post-Synaptic-Density-95 protein, a scaffolding protein that organizes proteins at the synapse[10].

ATP1A3 mutations and neurological disorders

In 1999, two large families were identified with multiple family members presenting with RDP.[13,14] Linkage analyses identified the 19q13 locus as the region most likely to harbor mutations associated with disease;[15] a finding that was confirmed in additional RDP families.[16,17] RDP in all families was inherited as an autosomal dominant trait with incomplete penetrance. Using a positional cloning approach, six different heterozygous missense mutations were identified in *ATP1A3* that co-segregated with the disease phenotype.[18] Since the initial discovery, a total of 11 mutations (Figure 2; Table 1; 9 missense mutations, a 3-bp in-frame deletion and a 3-bp in-frame insertion) have been reported in 20 RDP families, including 12 individuals with no family history of RDP.[17–31] Three RDP mutations, one found only in sporadic RDP cases and two found in both sporadic and familial RDP cases, are recurrent, most likely because they are located at hypermutable methylated CpG-dinucleotides in the *ATP1A3* gene (Table 1).[32]

In 2012, two independent studies, one performed by an international consortium[33] and one by German researchers,[34] identified *de novo* mutations in *ATP1A3* as the cause of AHC. In both studies next-generation sequencing (NGS) was used to screen the protein-coding portion of the genome of sporadic patients with AHC to look for disease-causing mutations that were absent in their unaffected parents. This approach identified a *de novo ATP1A3* mutation in each of the ten patients screened across the two studies, which definitively establishes *ATP1A3* as the first AHC gene.[33,34] This finding was later replicated by an

independent Japanese study that found *ATP1A3* mutations in eight out of ten patients with AHC.[35] The German study identified mutations in all 24 German patients with AHC. Notably, the international study reported *ATP1A3* mutations in 78% (82/105) of patients with AHC,[33] which suggests that some *ATP1A3* mutations may have been missed, that other AHC genes may exist, and/or that the diagnosis may not always be accurate. To date, 27 different *ATP1A3* mutations have been reported in patients with AHC (Figure 2, Table 1). Ten mutations have been identified in multiple individuals, with one mutation (D801N) explaining over 40% of AHC patients with an *ATP1A3* mutation (Table 1).

When considering the location in the ATP1A3 protein sequence of mutations that cause RDP and AHC an interesting difference emerges. Whereas RDP mutations seem to be spread across the protein, AHC mutations are located almost exclusively in particular regions of the protein (Figure 3). The significance of the different mutation patterns in RDP and AHC is currently unknown, but suggests that, unlike in RDP, only specific protein disruptions may result in AHC. In addition, rarely the same amino acid is mutated in RDP and AHC, but even in these cases the amino acid substitution is disease-specific (Table 1). There is only one RDP mutation (D923N) that also has been identified in an unusual case of familial AHC. In this multiplex AHC family, four individuals have the D923N mutation, including one with a diagnosis of AHC and three with some of the defining symptoms of AHC (see below).[29] This nearly perfect genotype-phenotype correlation, with a nearly non-overlapping set of mutations associated with AHC and RDP, strongly argues for a distinct functional effect of the mutations causing AHC and RDP, which is yet to be elucidated.

Consistent with mutations in *ATP1A3* causing neurodevelopmental diseases, only two polymorphic missense mutations (both with low population frequencies [minor allele frequency <;0•1%]) have been reported for *ATP1A3* (figure 2, table 1) in the Exome Variant Server, NHLBI GO Exome Sequencing Project (Seattle, WA, USA). The database houses variants from protein-coding genomes of approximately 6500 individuals who were not identified based on neurodevelopmental or neuropsychiatric disease phenotypes. Evaluating the relationship of the total number of polymorphic functional variants as a function of the total number of variants for each sequenced gene in the database indicates that observing two polymorphic functional variants in *ATP1A3* given the total number of variants reported is less that what is expected. Thus, *ATP1A3* is generally intolerant of functional variation in comparison with genome-wide expectations for a gene of its size and mutability, implying that individuals with functional mutations in this gene might be at high risk of developing serious diseases.[36]

Diseases caused by mutations in ATP1A3

There are two neurological disorders that are known to be associated with mutations in *ATP1A3*, RDP and AHC. Both diseases have been described independently, and have distinct, well-defined clinical criteria.

Rapid-onset dystonia-parkinsonism

Recognition of RDP began in 1993, more than ten years before *ATP1A3* was identified as the causal gene, when Dr. William B. Dobyns examined a 15-year-old girl that exhibited

abrupt onset of dystonia with prominent dysarthria and dysphagia.[14] The disorder was named RDP because of the abrupt onset of dystonic spasms associated with postural instability and bradykinesia that resemble signs of parkinsonism.[13,37–39] RDP is also sometimes referred to as Dystonia 12 (DYT12;OMIM: 128235). The clinical presentation of RDP includes:(1) its appearance which is often after triggering events such as running, alcohol binges, minor head injuries, overheating, emotional stress, infections, or childbirth;[21] (2) a rapid onset of typically permanent symptoms that develop over hours to days - occasionally even over a period of several weeks; and (3) involuntary movements that are characterized by generalized dystonia with superimposed parkinsonian features; primarily bradykinesia and postural instability without tremor. Many patients present with a rostro-caudal gradient of the dystonia and parkinsonism, in the sense that bulbar symptoms are more severe than arm symptoms, and arm symptoms being more severe than leg symptoms. The bulbar and arm symptoms rarely improve after the primary disease onset. A few patients reported later episodes of abrupt worsening of symptoms that occurred one to even nine years after the initial onset. One patient had transient symptoms after athletic activity, recovered, resumed strenuous athletic activity, and then had permanent onset of fixed symptoms. Not all patients report a recognized trigger, and a few report antecedent periods of cramping. Patients typically lack other disease features such as diurnal fluctuation or episodes that are typical of patients with AHC. RDP patients are unresponsive to standard medications for parkinsonism.[14,16,19,20,40] A recently published cohort of 26 RDP patients indicated that 76% of patients had onset of motor symptoms by the age of 25.[40] In addition to dystonia, there can also be non-motor manifestations in RDP patients. Recent work suggests that RDP patients may exhibit an elevated prevalence of mood disorders (50%) and psychosis (19%) compared to relatives without an *ATP1A3* mutation.[40] These findings were observed across families with different *ATP1A3* mutations, and are consistent with reports of depression in individuals with *ATP1A3* mutations causing motor problems.[16]

The originally published diagnostic criteria for RDP require a family history, an onset of the disease in teens, and prominent bulbar findings.[19] However, as the number of reported cases of RDP increases, these criteria appear too restrictive for several reasons: (1) more than half of RDP patients lack a positive family history as they are caused by *de novo* mutations in small, single-patient families; (2) disease onset has been reported also in children and adults;[30] and (3) there is a growing recognition that there are patients in which RDP may present with atypical features, including second onsets and unusual, mild to moderate improvement after the primary onset of disease symptoms (Panel A). Collectively, these findings suggest that the phenotype is broader than originally described in 1993. Drug therapy in RDP is limited. RDP patients are unresponsive to standard medications for parkinsonism, including levodopa.[14,16,19,20,40] Current treatment is limited to benzodiazepines that have been reported to provide symptomatic relief in some patients. *ATP1A3* is the only known RDP gene; however, other RDP genes may exist as evidenced by one study reporting that, in a select group of 14 patients referred for possible RDP, only three were found to have a mutation in *ATP1A3*.[19]

Alternating hemiplegia of childhood

The most widely accepted clinical criteria to diagnose AHC, as proposed by Bourgeois and co-workers,[41] are commonly known as the "Aicardi Criteria" (summarized in Panel A). Over the years patients with AHC have been subjected to many treatments to alleviate the frequency and severity of hemiplegia, although their success has been very limited.[42,43] Of them, flunarizine, a calcium channel blocker, came up best as it seems to reduce the severity and duration of the attacks, at least in some patients.[44–47] Benzodiazepines, which act to increase the activity of γ-aminobutyric acid (GABA), the major inhibitory neurotransmitter in the central nervous system, may also have some efficacy either directly or by inducing sleep that often relieves attacks.[42,43]

While nearly all cases of AHC are sporadic, there are some families with an autosomal dominant inheritance of AHC.[48–51] In two families a causal *ATP1A3* mutation was identified,[29,33,51] and in one family a disease-causing *ATP1A2*(encoding the α2 subunit of the Na, K-ATPase) mutation was found.[49,50] Consistent with these mutations being highly penetrant, all *ATP1A2* or *ATP1A3* mutation carriers in these families have symptoms of AHC, albeit to varying severity even within families.[29,3349–51] In addition to these unusual familial cases, there are also patients with atypical presentations that resemble AHC, such as Benign Nocturnal Alternating Hemiplegia that occurs only in boys,[52,53] mild cases with normal cognitive development,[54] cases in whom dystonia is the predominant feature,[54] patients who do not have episodes of quadriplegia, and patients who experience the first signs of the disorder after the age of 18 months,[54,55] patients presenting with neonatal seizures, and patients with status epilepticus with associated long-term atrophy on the MRI and residual motor and eye movement abnormalities.[56] The role of *ATP1A3* mutations in these conditions that are somewhat related to AHC is not yet known.

Genotype-phenotype correlation

While the number of AHC and RDP patients with an identified *ATP1A3* mutation is rapidly growing, definitive phenotypic patterns have not been found for patients with and without mutations, and among patients with recurring *ATP1A3* mutations.[33,34,57]. However, one small study evaluating the phenotypes of 35 AHC patients with *ATP1A3* mutations, reports that patients with the E815K mutation tend to have earlier onset of symptoms, more severe motor and cognitive disabilities, and more often report status epilepticus and respiratory paralysis compared to AHC patients with other *ATP1A3* mutation.[58] This preliminary finding suggests that genotype-phenotype correlations may exist and that additional studies will be needed to further evaluate these patterns in larger sample sizes. The ATP1A3 working group is currently analyzing 150 patients for genotype-phenotype correlations.

Panel A

Proposed diagnostic criteria for AHC and RDP[13,14,16,19,20,29,30,33,37–50,52–56,59–61]

	AHC	RDP
Age of Onset	0–18 months (median 3.5 months)	Can occur in children and adults (9 months to 59 years)

	AHC	RDP
	Isolated cases although there are some later onset cases as reported as late as 4yrs; median 3.5 months)	76% of patients report symptoms by age 25
Inheritance	Typically sporadic Rare cases of familial AHC	Sporadic or familial
Clinical Presentation	Paroxsymal symptoms: Episodes of hemiplegia, bilateral hemiplegia, or quadriplegia with improvement in sleep and with other paroxysmal abnormalities such as dystonia, tonic spells, epileptic seizures, autonomic changes and/or abnormal eye movements often with the following characteristics: Common triggers include stress, excitement, extreme heat or cold, water exposure, physical exertion, lighting changes, and foods Variable frequency ranging from multiple episodes per day to one every few months Associated with anarthria, dysphagia, and autonomic disturbances including bradycardia, stridor, bronchospasm, apnea, dyspnea, nausea, unilateral or bilateral flushing, hypo- or hyperthermia Episodes typically remit with sleep but may return within 10–20 min after waking Associated with abnormal eye movements including unilateral (ipsilateral to the hemiplegia) intermittent eye deviation, disconjugate gaze, and pendular nystagmus Episodes of dystonia in concert or separate from episodes of hemiplegia, bilateral hemiplegia, quadriplegia, or abnormal eye movements that are more commonly observed early in the clinical course, typically consist of head turning toward the affected side with eye deviations toward the same side-Very brief intense unilateral or bilateral tonic/dystonic attacks with vibratory tremor and pain Seizures occur in about 50% of patients and can include focal or generalized tonic, tonic-clonic, or myoclonic seizure types Interictal symptoms: Evidence of developmental delay, mental retardation and interictal neurologic abnormalities such as tone abnormalities, choreoathetosis, and/or ataxia: Choreoathetosis- spontaneous or movement induced Ataxia that is sometimes associated with cerebellar atrophy or cerebellar hypometabolism Developmental delay including intellectual deficiencies, neuopsychological deficits, Persistent motor deficits such as spastic diplegia/quadriplegia or hypotonia	Typically abrupt onset of permanent symptoms of generalized dystonia and parkinsonian features including bradykinesia and postural instability Variable progression of symptoms ranging from hours to weeks Diurnal fluctuation or episodes typical of patients with AHC are not seen in RDP Abrupt worsening of symptoms may rarely occur years later after initial onset Onset of symptoms typically occurs after running, alcohol binges, minor head injuries, overheating, emotional stress, infections, or childbirth May be associated with mood disorders and psychosis
Drug Therapy	Flunarizine Benzodiazepines	Unresponsive to levodopa

Biological consequences of disease-causing ATP1A3 mutations

In vitro studies

Effects on ATP1A3 protein expression and localization—Ten RDP and five AHC *ATP1A3* mutations have been investigated at the level of protein expression and/or cellular localization of the protein by using heterologous expression systems.[18,33] These studies revealed that for all except two of the tested RDP mutations the expression level of ATP1A3 protein was reduced, whereas none of the AHC mutations reduced expression of ATP1A3. RDP mutations were not shown to affect the maturation and localization of the protein in transfected cells;[18] the effect of AHC mutations on ATP1A3 localization has not yet been studied.

Effects of ATP1A3 mutations in platelets and fibroblasts—Platelets and fibroblasts from 9 AHC patients have been screened for differences in protein expression compared to age- and sex-matched controls.[62] A consistent change was observed in lysosomal protein cathepsin in AHC patient specimens which was shown to increase apoptosis. While the mechanism remains unclear, this work suggests that similar protein changes may also occur in the brain and may contribute to AHC pathophysiology.

Effects on Na, K-ATPase activity—In the established Post-Albers model for the Na, K-ATPase transport mechanism (Figure 4),[63] in which three cytoplasmic Na^+ ions are exchanged for two extracellular K^+ ions for each ATP being hydrolyzed, the E1 conformation preferentially binds Na^+, whereas E2 prefers K^+. Based on this model, there are several approaches to experimentally measure the activity of the Na, K-ATPase, and specifically the effects of disease-causing mutations in the *ATP1A3* on the catalytic cycle.

First, using a luminescent kinase assay that measures ADP formed from each catalytic cycle of the Na, K-ATPase (Figure 4) from cells heterologously expressing the wild-type, RDP-, or AHC-causing mutant version of the *ATP1A3* cDNA, each mutation was shown to reduce the activity of the Na, K-ATPase.[33] These findings, coupled with the aforementioned protein expression analyses, suggest that mutations that affect Na, K-ATPase functioning, but not the amount of Na, K-ATPase *per se*, seem to result in the more severe AHC phenotype.

Second, since an active Na, K-ATPase pumps a net charge of +1 out of the cell for each round of the catalytic cycle (Figure 4), electrophysiology-based approaches can be used to measure in a living cell or in a patch of excised membrane the voltage and ion sensitivity of the pump during steady-state activity. Two disease-causing *ATP1A3* mutations have been characterized in considerable detail: D801N and D923N. The aspartate located at amino acid position 801 is highly conserved in Na, K-ATPases among all investigated species, and is predicted to act as a site that binds either the Na^+ or K^+ ions during the catalytic cycle. Expression of the AHC-causing D801N mutant in *Xenopus* oocytes showed that the mutation generated no measurable pump current, consistent with it being unable to bind K^+ ions.[64] Analysis of the D923N mutation, which was identified in both patients with RDP[23,25,29,30] and familial AHC,[29] suggested that protonation at this site was critical for the movement of Na^+ and K^+ across the cell membrane.[65]

Third, during the ion transport process, the Na, K-ATPase becomes phosphorylated by transfer of the γ-phosphate of ATP to a conserved aspartic acid residue in the P-type ATPase signature sequence (Figure 4). By incubating ATP, radio labelled at the γ-phosphate, with cell membrane fragments containing the Na, K-ATPase to quantify this covalent and acid-stable enzyme phosphoryl bond, the activity of the Na, K-ATPase can be measured with and without disease-causing mutations.[66–69] Because the binding of three Na^+ ions at the cytoplasmic oriented surface of the ATP1A3 protein is required to activate the enzyme for phosphorylation from ATP, the affinity for Na^+ at these sites can be determined by studying the Na^+ dependence of phosphorylation. So far, RDP-causing mutations E277K, T613M, F780L, and +Tyr (that is an extension of the C-terminus with an extra Tyr residue), and RDP/AHC-causing mutation D923N, have been characterized using these methodologies.[24,66–68] Each of the studied mutations exhibit marked reductions of Na^+ affinity for activation of phosphorylation. While Na^+ binding from the cytoplasmic side activates phosphorylation from ATP, binding of K^+ from the external side triggers de-phosphorylation, thereby stimulating ATP hydrolysis. Therefore, the affinity for external K^+ can be determined by studying the K^+ dependence of de-phosphorylation or ATPase activity at a fixed Na^+ concentration. Notably, none of the mutants show a reduced affinity for K^+, indicating a selective disturbance of Na^+ binding that is associated with RDP- and AHC-causing mutations.[66–69]

The above described studies show that a selective reduction of the affinity of the Na, K-ATPase for cytoplasmic Na^+ without a disturbance of K^+ binding is a central theme in RDP. Consequently, an increased intracellular Na^+ concentration resulting from the reduced Na^+ affinity may be a central pathogenic factor in RDP.[69] A rise in intracellular Na^+ may result in a secondary increase in intracellular Ca^{2+} via the Na^+ /Ca^{2+} exchange system, which can activate signaling cascades triggered by changes in Ca^{2+} concentration. In addition, it is possible that a disturbance of the Na^+ gradient will affect the uptake of neurotransmitters, such as dopamine and glutamate. Because several AHC-causing mutations target the same residue as found mutated in RDP, an essential question to address is whether Na^+ affinity is also disturbed in AHC. The affinity for K^+ may also be disturbed in AHC, which may also explain why AHC is at the severe end of the phenotypic spectrum whereas RDP is at the mild end. Structural modeling of three AHC-causing mutations (I274N, D801N, D923Y) and three RDP-causing mutations (I274T, D801Y, D923N) that affect identical positions in the Na, K-ATPase α3 subunit[70] predicted that AHC mutations would bring about structural changes that severely affect an efficient K^+ movement along the narrow K^+ access pathway. Instead, RDP-causing mutations seem to have milder structural consequences that are likely to result in a milder impairment of K^+ conductance.[71]

Notably, a mutation in the sarco-endoplasmic reticulum Ca^{2+} ATPase, a member of the same Type II P-type ATPases family as the Na, K-ATPases, in *Drosophila melanogaster*, was recently shown to cause temperature-sensitive ionic leakage of the transporter[72]. It was postulated that a temperature-sensitive gain-of-function mechanism might also underlie the phenotypic consequences of disease-causing mutations in other Type II P-type ATPases, including *ATP1A3* that is mutated in AHC and RDP. While the impact of temperature on the functional effects of AHC-and RDP-causing mutations in Na, K-ATPases is unknown, if

correct, this could explain why environmental triggers like stress, physical exertion and temperature changes may lead to symptom onset in patients with RDP and AHC (Panel A).

Animal studies

A number of different animal models have been used to study the *in vivo* consequences of ATP1A3 modulation (Figure 5).

Myshkin mice—The amino acid sequence identity between the human and mouse Na, K-ATPase α3 subunits is about 99%. Heterozygous *Myshkin* (*Atp1a3*$^{Myk/+}$; *Myk*/+) mutant mice carry an amino acid change (I810N) that affects the identical position as I810S in the human Na, K-ATPase α3 subunit that was identified in a patient with AHC.[73,74] Molecular modeling of I810N and I810S showed that both bring about similarly severe structural impacts on the Na, K-ATPase α3 subunit, including the capacity for an efficient K^+ movement along the K^+ access pathway.[71] I810N was generated through *N*-nitroso-*N*-ethylurea (ENU) mutagenesis and results in a normally expressed, but inactive, α3 protein, and a subsequent 36–42% reduction in total Na, K-ATPase activity (reflecting the activity of α1, α2 and α3 combined) in the brain.[73,75] Heterozygous *Myk*/+ mice exhibit an unsteady, tremulous gait with occasional splaying of the hindlimbs but without an overt hemiplegia.[71] Phenotypic analysis revealed a range of other abnormalities in *Myk*/+ mice, including a reduction in body size; motor deficits in the balance beam and rotarod tests; cognitive deficits in the fear-conditioning and conditioned taste aversion tests; neuronal hyperexcitability with spontaneous convulsions; and mania-related behaviors, such as increased risk-taking and responsiveness to treatment with lithium and valproic acid.[71,73,75] When subjected to vestibular stress, *Myk*/+ mice exhibit transient tonic attacks and staggering movements that, in one-third of mice, develop into tonic-clonic seizures that are accompanied by epileptiform discharges.[73] [^{14}C]-2-deoxyglucose imaging of *Myk*/+ mice identified compromised thalamocortical functioning, including a deficit in frontal cortex functioning and reduced thalamocortical functional connectivity.[71] When bred to homozygosity, *Myk/Myk* pups initially appear grossly normal but die shortly after birth.[73] Treatments aimed at increasing Na, K-ATPase activity have shown therapeutic effects in *Myk*/+ mice. This nicely fits findings from transgenic delivery of an additional copy of the wild-type *Atp1a3* gene to the X chromosome, which increased Na, K-ATPase α3 subunit protein expression and whole brain Na, K-ATPase activity, and reduced the epileptic seizure susceptibility as well as the risk-taking behavior of *Myk*/+ mice.[73,76] Chronic treatment with rostafuroxin, a compound that antagonizes the inhibitory action of ouabain on Na, K-ATPase,[77] was also found to reduce the risk-taking behavior of *Myk*/+ mice.[75] Effects of this intervention on the motor and cognitive deficits of *Myk*/+ mice have not yet been determined.

Atp1a3^{tm1Ling} mice—Heterozygous *Atp1a3*$^{tm1Ling/+}$ mice, which carry a point mutation in intron 4 of the *Atp1a3* gene, show a reduction of hippocampal α3 protein expression of ~60% and a reduction of total brain Na, K-ATPase activity (of α1, α2 and α3 combined) of ~16%.[73,76,78] Non-stressed (naïve) *Atp1a3*$^{tm1Ling/+}$ mice do not exhibit visible neurological defects or restricted growth, but instead show increased locomotor activity in an open field test and deficient spatial learning in the Morris water maze test.[78] After exposure to restraint

stress for five days, female *Atp1a3*$^{tm1Ling/+}$ mice exhibit mild motor deficits in the balance beam and rotarod tests.[79] *Atp1a3*$^{tm1Ling/+}$ mice exposed to chronic variable stress, consisting of one or two unpredictable mild stressors per day for 6 weeks, exhibit deficits in total brain Na, K-ATPase activity, sociability and object recognition memory, as well as increased anxiety and depression-like behaviors, compared to non-stressed *Atp1a3*$^{tm1Ling/+}$ mice.[76] In wild-type mice, chronic variable stress also led to depression-like behavior and less sociability, but had no effect on Na, K-ATPase activity, anxiety or object recognition memory, when compared to non-stressed wild-type controls.[76] Homozygous *Atp1a3*^{tm1Ling} pups die shortly after birth.[78]

Atp1a3^{tm2Kwk} mice—Heterozygous *Atp1a3*$^{tm2Kwk/+}$ mice carry a targeted deletion of *Atp1a3* exons 2–6.[80] *Atp1a3*$^{tm2Kwk/+}$ mice do not exhibit gross morphological defects or apparent histological brain anomalies. Adult male *Atp1a3*$^{tm2Kwk/+}$ mice show increased locomotor activity, both in the home cage and in the open field test. In contrast to *Atp1a3*$^{Myk/+}$ mice, performances of *Atp1a3*$^{tm2Kwk/+}$ mice in the balance beam and rotarod tests were enhanced compared to wild-type mice. *Atp1a3*$^{tm2Kwk/+}$ mice do not develop dystonia spontaneously, nor after various stressors, such as tail suspension, forced swimming, or restraint. Dystonia can be induced pharmacologically,[81] in these mice by injecting the neuro-excitatory amino acid kainate (KA) directly into the cerebellum. The response to dystonia induction by KA injection was found increased in *Atp1a3*$^{tm2Kwk/+}$ mice with a longer duration of sustained dystonia compared to wild-type mice. Electrophysiological studies showed that inhibitory neurotransmission at molecular-layer interneuron Purkinje cell synapses was enhanced in the cerebellar cortex of *Atp1a3*$^{tm2Kwk/+}$ mice. Homozygous *Atp1a3*^{tm2Kwk} mice show a complete lack of breathing movements and die just after birth.[80]

Pharmacological blockade of Na, K-ATPase α3—Another mouse model employs a pharmacological approach to perturb Na, K-ATPase function. Perfusion of the Na, K-ATPase inhibitor ouabain into the cerebellum and basal ganglia was found to induce mild dyskinesia in wild-type C57BL/6 mice.[82] When mice were subsequently exposed for 2 hours to stress provided in the form of random electric foot shocks in a warm environment (38°C), 70% of the mice developed persistent dystonia and rigidity[82]. These mice exhibit hallmark symptoms of RDP, including the dystonia and parkinsonism induced by stress. However, this approach is limited by the similar affinities of the α2 and α3 isoforms for ouabain,[83] thus precluding α3 specificity in this animal model.

Zebrafish—Zebrafish (*Daniorerio*) have two *ATP1A3* orthologues, *Atp1a3a* and *Atp1a3b*.[84] The paralogous α3a and α3b subunits have amino acid identities of 95% with each other and 91–92% with the human Na, K-ATPase α3 subunit protein sequence. Consistent with mammalian Na, K-ATPase α3 subunit protein expression, the transcripts of *Atp1a3a* and *Atp1a3b* are primarily expressed in the brain, albeit with distinct expression profiles. In 60h post-fertilization (hpf) zebrafish embryos, the *Atp1a3a* transcript is widely distributed throughout the brain, whereas distribution of *Atp1a3b* mRNA is more localized to particular brain structures. Despite having distinct expression profiles, targeted knockdown (KD) of *Atp1a3a* or *Atp1a3b* by morpholino antisense oligonucleotides results

in severe brain ventricle dilation in 60hpf embryos, suggesting that both α3 paralogues are required for brain ventricle maintenance. The extent of brain ventricle dilation was reduced by co-injection of the mRNA of the knocked-down gene, but *Atp1a3b* mRNA did not cross-rescue the phenotype of *Atp1a3a*-KD embryos. Similarly, *Atp1a3a* mRNA did not cross-rescue the phenotype of *Atp1a3b*-KD embryos. Both morphants display abnormal spontaneous motility and an abnormal response to tactile stimulation with a needle,[85] suggesting that both α3 paralogues are required for embryonic motility.

Drosophila—The gene *Atp*α (FlyBase ID: FBgn0002921) in *Drosophila melanogaster* fruit flies encodes the α subunit of the Na, K-ATPase, which is orthologous to all vertebrate α subunits, and has amino acid sequence identities of 76–77% with the α1, α2 and α3 subunits of humans.[86] Although the *Drosophila Atp*α gene is not a specific orthologue of *ATP1A3*, eight missense mutations, generated through ethylmethanesulfonate (EMS) mutagenesis at highly conserved amino acid residues, lead to AHC-relevant phenotypic abnormalities in adult heterozygous flies.[87,88] Flies from any of six mutant lines with mutations (S201L, P262L, S348T, G528S, A588T, G744S) in *Atp*α that were repeatedly knocked to the bottom of a vial using a standard laboratory vortexer exhibited transient mechanical stress-induced paralysis.[88] Two other mutant lines (D981N, E928K) did not exhibit this phenotype when maintained at an ambient temperature of 20–22°C, but showed temperature-sensitive mechanical stress-induced paralysis only when maintained at 28°C.[87] When exposed to a temperature of 37–38°C, three mutant lines (G744S, D981N, E928K) exhibited temperature-sensitive paralysis that was reversed when the ambient temperature was lowered to 20–22°C.[87,88] When the body weight of male flies of six of the same mutant lines was determined, only S201L mutant flies showed a reduction compared with wild-type flies.[88] Western blotting of homogenized fly heads showed that the abundance of Na, K-ATPase α subunit was reduced in two of the *Atp*α mutants (S348T, A588T) but unaltered in the other four mutants.[88] Mutations G744S and D981N in the *Drosophila* α subunit, which led to temperature-sensitive paralysis, affect equivalent amino acid residues in the human Na, K-ATPase α3 subunit, namely mutations G755C, G755S and D992Y, which were identified in patients with AHC (Table 2).[34,74] All eight missense mutations are homozygous lethal.[88]

Conclusions

Since the original recognition of RDP and AHC as diseases, substantial work has been performed to characterize their clinical presentation and pathophysiology. Through the identification of disease-causing mutations in the *ATP1A3* gene, these two seemingly unrelated diseases are now linked, allowing new possibilities to obtain insight into their biological bases and instigating many novel areas of investigation.

It is now understood that protein-modifying genetic variation in *ATP1A3* rarely occur in the general population, and when they do, the risk of developing a severe neurological disease is very high. This has led to an important new research area, investigating what other related diseases may be associated with mutations in *ATP1A3*. One may postulate that *ATP1A3* mutations may also be found in patients with seizures, psychiatric conditions, or other less severe types of dystonia or ataxia. As NGS becomes more widely used in day-to-day clinical

practice, the role of *ATP1A3* mutations in a wider range of phenotypes, if they exist, will become apparent soon. However, while this genotype-phenotype spectrum is being defined, we can already begin cataloging *ATP1A3* disease-specific mutations and polymorphic protein-disrupting variants in the general population, to establish molecular and physiologic changes associated with these DNA variations in *in vitro* and *in vivo* test paradigms like those described in this review. Data that will emerge from these studies can be used to develop or fine-tune better research models with tiered complexity. We envisage that these studies will include establishing disease models at the transporter level in individual cells, multicellular network models using induced pluripotent cells differentiated into neurons, *ex vivo* studies in brain slices to study tissue level consequences, and, finally, evaluations at the organismal level to assess *in vivo* consequences in animal models. With these more developed model systems we can begin to try to relate molecular changes to the unique phenotypic presentation associated with these disorders, including the episodic nature of AHC, how particular stimuli lead to the onset of symptoms, the age-dependent onset of RDP, and the variable effects on organ systems and brain structures that likely underlie the diverse phenotypic presentations.

Importantly, once we identify key biomarkers of disease pathophysiology, we will be able to identify and screen compounds to rectify the pathophysiological changes associated with *ATP1A3* mutations.

In summary, genetics has illuminated key aspects of disease pathophysiology for both AHC and RDP. While extensive work is needed to disentangle the complex biology underlying these disorders, we are poised with evolving research approaches to rapidly translate these genetic discoveries to detailed disease pathophysiology, to improved understanding of developmentally-mediated and environmentally-triggered disease presentation, and ultimately to identify and develop better treatments for these debilitating diseases.

Acknowledgments

We would like to kindly acknowledge the support of the European Network for Research on Alternating Hemiplegia, the members of the NEUROPED project, and Duke University for organizing the first meeting of the ATP1A3 Working Group in Brussels, Belgium in December 2012. This review arose from the discussions and presentations at that meeting. Other financial contributors of this meeting including: RTD Services, Austria, French Association for Alternating Hemiplegia (AFHA), Icelandic Association for Alternating Hemiplegia (AHCAI), Italian Association for Alternating Hemiplegia (A.I.S.E.A Onlus), Spanish Association for Alternating Hemiplegia (AESHA), Dutch Association for Alternating Hemiplegia (AHC Vereniging Nederland), German Association for Alternating Hemiplegia (AHC-Deutschland e.V.), US Foundation for Alternating Hemiplegia (AHCF). We would also like to thank specific individuals who contributed to and supported the efforts of the ATP1A3 Working Group, including: Sharon Ciccodicola (AHCF), Thierry Billette de Villemeur, Lynn Egan (AHCF), NardoNardocci, Anne Roubergue, Francesca Ragona, Shoji Tsuji, Rosaria Vavassori, and Federico Vigevano.

In addition, some of the work presented in this review was supported by the Ontario Mental Health Foundation Research Studentship, MOP 94856 from the Canadian Institutes of Health Research, and a grant from the Amalgamated Transit Union.

Artwork shown in Figure 5 was produced by Samantha Sliwa.

ATP1A3 Working Group

Alexis Arzimanoglou[3,26], Frances M. Ashcroft, Ph.D.[27], Allison Brashear, M.D.[5], Knut Brockmann, M.D.[28], Jaume Campistol, M.D.[29], Alessandro Capuano, Ph.D.[30], Inês

Carrilho, M.D.[31], Paul Casaer[32], Steven J. Clapcote, Ph.D.[6], Elisa De Grandis, Ph.D.[33], Boukje de Vries, Ph.D.[23], Michela Di Michele, Ph.D.[34], Caroline Dion[35], Diane Doummar, M.D.[36], Anja P. Einholm, Ph.D.[25], Carmen Fons, M.D.[29], Filippo Franchini[9,37], Thomas Friedrich, Ph.D.[38], Kathleen Freson, Ph.D.[34], David C. Gadsby, Ph.D.[39], Melania Giannotta, M.D.[40], David B. Goldstein, Ph.D.[1,8], Christophe Goubau, Ph.D.[34,41], Titiana Granata, M.D.[42], Fiorella Gurrieri, Ph.D.[7], Erin L. Heinzen, Ph.D.[1,2], Shinichi Hirose, M.D.[43,44], Yuki Hitomi, Ph.D.[1], Rikke Holm, M.Sc.[25], Keiko Ikeda[45], Atsushi Ishii, Ph.D.[43,44], Sigurður H. Jóhannesson[9,10], Kamran Khodakhah, Ph.D.[46], Mary D. King, FRCPCH[47], Greer S. Kirshenbaum, Ph.D.[48,49], Ana Kockhans[50], Jan B. Koenderink, Ph.D.[51], Gaetan Lesca, M.D.[4,52,53], Karin Lykke- Hartmann, Ph.D.[20,25], Ulrike Maschke, M.D.[54], Mario R. Merida, M.D.[55], Mohamad A. Mikati, M.D.[11,12], Ralf Müller[50], Giovanni Neri, M.D.[7], Brian Neville, FRCPCH[13], Sophie Nicole, Ph.D.[14–16], Hang N. Nielsen, M.Sc.[25], Poul Nissen, Ph.D.[19,20], Tom O'Brien[56], Laurie J. Ozelius, Ph.D.[17,18], Eleni Panagiotakaki, M.D.[3], Marek Parowicz[57], Dominique Poncelin[58], Hanne Poulsen, Ph.D.[19,20], Sandra P. Reyna, M.D.[59], John C. Roder, Ph.D.[48,49], Hendrik Rosewich, M.D.[60], Masayuki Sasaki, M.D.[61], Vivien R. Schack, Ph.D.[25], Philippe Schyns, Ph.D.[21], Tsveta Schyns, Ph.D.[21], Michela Stagnaro, Ph.D.[33], Kathleen J. Sweadner, Ph.D.[22], Kathryn J. Swoboda, M.D.[59,62], Danilo Francesco Tiziano, Ph.D.[7], Mads S. Toustrup-Jensen, Ph.D.[25], Arn van den Maagdenberg, Ph.D.[23,24], Albert Vilamala, M.S.[9,63], Bente Vilsen, D.M.Sc.[25], Jeff T. Wuchich, B.A.[64]

1 Center for Human Genome Variation, Duke University, School of Medicine, Durham, NC 27708, USA.

2 Department of Medicine, Section of Medical Genetics, Duke University, School of Medicine, Durham, NC 27708, USA.

3 Epilepsy, Sleep and Pediatric Neurophysiology Department, HFME, University Hospitals of Lyon (HCL), Lyon, France.

4 CRNL, CNRS UMR 5292, INSERM U1028, Lyon, France.

5 Department of Neurology, Wake Forest School of Medicine, Winston Salem, NC 27157, USA.

6 School of Biomedical Sciences, University of Leeds, Leeds LS2 9JT, UK.

7 Istituto di Genetica Medica, Università Cattolica S. Cuore, Rome, Italy.

8 Department of Molecular Genetics and Microbiology, Duke University School of Medicine, Durham, North Carolina, USA.

9 AHC Federation of Europe (A.H.C.F.E.) - Jórusel 18, 109, Reykjavík, Iceland.

10 AHC Association of Iceland, Reykjavik, Iceland.

11 Division of Pediatric Neurology, Duke University Medical Center, Durham, North Carolina, USA.

12 Department of Neurobiology, Duke University, Durham, North Carolina, USA.

13 Institute of Child Health, University College London, London, UK.

14 Institut National de la Santé et de la Recherche Médicale, U975, Centre de Recherche de l'Institut du Cerveau et de la Moelle, Paris, France.

15 Centre National de la Recherche Scientifique, UMR7225, Paris, France.

16 Université Pierre et Marie Curie Paris VI, UMRS975, Paris, France.

17 Department of Genetics and Genomic Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

18 Department of Neurology, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

19 Danish Research Institute for Translational Neuroscience, Nordic-EMBL Partnership of Molecular Medicine, Department of Molecular Biology and Genetics, Aarhus University, Denmark.

20 Centre for Membrane Pumps in Cells and Disease-PUMPKIN, Danish National Research Foundation, Denmark.

21 European Network for Research on Alternating Hemiplegia (ENRAH).

22 Neurosurgery, Massachusetts General Hospital, Boston, Massachusetts, USA.

23 Department of Human Genetics, Leiden University Medical Centre, P.O. Box 9600, 2300 RC Leiden, The Netherlands.

24 Department of Neurology, Leiden University Medical Centre, P.O. Box 9600, 2300 RC Leiden, The Netherlands.

25 Department of Biomedicine, Aarhus University, Denmark.

26 Centre de Recherche en Neurosciences de Lyon, Centre National de la Recherche Scientifique, UMR 5292; Institut National de la Santé Et de la Recherche Médicale, U1028, Lyon, France.

27 Department of Physiology Anatomy & Genetics, University of Oxford, Parks Road, Oxford OX1 3PT, UK.

28 Interdisciplinary Pediatric Center for Children with Developmental Disabilities, University Medical Center, Göttingen, Germany.

29 Department of Pediatric Neurology. Hospital Sant Joan de Déu- Barcelona University. Barcelona, Spain.

30 Division of Neurology, Bambino Gesù Pediatric Hospital, IRCSS, Rome.

31 Department of Pediatrics, Division of Pediatric Neurology, Hospitalar Center of Oporto, Oporto, Portugal.

32 Department of Pediatric Neurology, University Hospital Gasthuisberg, Leuven, Belgium.

33 Child Neuropsychiatry Unit. Department of Neurosciences, Rehabilitation, Ophtalmology, Genetics and Maternal and Children's Sciences. G. Gaslini Institute, University of Genoa, Largo Gaslini 5, 16148 Genoa, Italy.

34 Department of Cardiovascular Sciences, Centre for Molecular and Vascular Biology, KU Leuven, Leuven, Belgium.

35 Canadian Association for Alternating Hemiplegia, Saint-Mathieu de Beloeil, Québec, Canada.

36 AP-HP, Service de Neuropédiatrie, Hôpital Trousseau, Paris, France. Centre de référence des mouvements anormaux de l'enfant à l'adulte.

37 A.I.S.EA Onlus (The Italian AHC Association), Via Sernovella, 37, I-23878 Verderio Superiore (LC), Italy.

38 Technical University of Berlin, Institute of Chemistry Sekr. PC 14, Straße des 17. Juni 135, D-10623 Berlin, Germany.

39 Laboratory of Cardiac/Membrane Physiology, Rockefeller University, New York, NY 10065, USA.

40 Child Neurology Unit, IRCCS Istituto delle Scienze Neurologiche di Bologna, Italy.

41 Department of Pediatrics, University Hospital Leuven, Leuven, Belgium.

42 Department of Pediatric Neuroscience, National Neurological Institute C.Besta, Milan, Italy.

43 Department of Pediatrics, School of Medicine, Fukuoka University, Fukuoka, Japan

44 Central Research Institute for the Molecular Pathomechanisms of Epilepsy, Fukuoka University, Fukuoka, Japan.

45 Division of Biology Hyogo, College of Medicine, Japan.

46 Albert Einstein College of Medicine, New York, US.

47 The Childrens University Hospital Temple St, Dublin 1, Ireland.

48 Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, Canada M5G 1X5.

49 Institute of Medical Science, University of Toronto, Toronto, ON, Canada M5S 1A8.

50 AHC-Deutschland e.V., Germany.

51 Department of Pharmacology and Toxicology 149, Radboud University Nijmegen Medical Centre, P.O. Box 9101, 6500 HB Nijmegen, The Netherlands.

52 Department of Genetics, University Hospitals of Lyon, Lyon, France.

53 Claude Bernard Lyon I University, Lyon, France.

54 Catholic Hospital St Johann Nepomuk, Erfurt, Germany.

55 Dean of the College of Allied Health, Stevens-Henager College, Ogden, Utah 84409, USA.

56 Alternating Hemiplegia of Childhood Ireland, Dublin, Ireland.

57 Polish AHC Association, Poland.

58 French AHC Organisation AFHA.

59 Department of Neurology, University of Utah School of Medicine, Salt Lake City, UT 84132, USA.

60 Department of Pediatrics and Pediatric Neurology, University Medical Center, Georg August University Göttingen, Germany.

61 Department of Child Neurology, National Center of Neurology and Psychiatry, Kodaira, Tokyo 187-8551, Japan.

62 Department of Pediatrics, University of Utah School of Medicine, Salt Lake City, UT 84132, USA.

63 AHC Association of Spain (AESHA).

64 Cure AHC, Rolesville, NC 27571, USA.

References

1. Boycott KM, Vanstone MR, Bulman DE, MacKenzie AE. Rare-disease genetics in the era of next-generation sequencing: discovery to translation. Nat Rev Genet. 2013; 14(10):681–691. [PubMed: 23999272]
2. McGrail KM, Phillips JM, Sweadner KJ. Immunofluorescent localization of three Na, K-ATPase isozymes in the rat central nervous system: both neurons and glia can express more than one Na, K-ATPase. J Neurosci. 1991; 11(2):381–391. [PubMed: 1846906]
3. Bottger P, Tracz Z, Heuck A, Nissen P, Romero-Ramos M, Lykke-Hartmann K. Distribution of Na/K-ATPase alpha 3 isoform, a sodium-potassium P-type pump associated with rapid-onset of dystonia parkinsonism (RDP) in the adult mouse brain. J Comp Neurol. 2011; 519(2):376–404. [PubMed: 21165980]
4. Dobretsov M, Stimers JR. Neuronal function and alpha3 isoform of the Na/K-ATPase. Frontiers in bioscience : a journal and virtual library. 2005; 10:2373–2396. [PubMed: 15970502]

5. Dobretsov M, Hastings SL, Sims TJ, Stimers JR, Romanovsky D. Stretch receptor-associated expression of alpha 3 isoform of the Na+, K+-ATPase in rat peripheral nervous system. Neuroscience. 2003; 116(4):1069–1080. [PubMed: 12617948]
6. Crambert G, Hasler U, Beggah AT, et al. Transport and pharmacological properties of nine different human Na, K-ATPase isozymes. J Biol Chem. 2000; 275(3):1976–1986. [PubMed: 10636900]
7. Senatorov VV, Mooney D, Hu B. The electrogenic effects of Na(+)-K(+)-ATPase in rat auditory thalamus. J Physiol. 1997; 502(Pt 2):375–385. [PubMed: 9263917]
8. Vaillend C, Mason SE, Cuttle MF, Alger BE. Mechanisms of neuronal hyperexcitability caused by partial inhibition of Na+-K+-ATPases in the rat CA1 hippocampal region. J Neurophysiol. 2002; 88(6):2963–2978. [PubMed: 12466422]
9. Shiina N, Yamaguchi K, Tokunaga M. RNG105 deficiency impairs the dendritic localization of mRNAs for Na+/K+ ATPase subunit isoforms and leads to the degeneration of neuronal networks. J Neurosci. 2010; 30(38):12816–12830. [PubMed: 20861386]
10. Blom H, Ronnlund D, Scott L, et al. Spatial distribution of Na+-K+-ATPase in dendritic spines dissected by nanoscale superresolution STED microscopy. BMC Neurosci. 2011; 12:16. [PubMed: 21272290]
11. Rose CR, Konnerth A. NMDA receptor-mediated Na+ signals in spines and dendrites. J Neurosci. 2001; 21(12):4207–4214. [PubMed: 11404406]
12. Reinhard L, Tidow H, Clausen MJ, Nissen P. Na(+), K (+)-ATPase as a docking station: protein-protein complexes of the Na(+), K (+)-ATPase. Cell Mol Life Sci. 2013; 70(2):205–222. [PubMed: 22695678]
13. Brashear A, DeLeon D, Bressman SB, Thyagarajan D, Farlow MR, Dobyns WB. Rapid-onset dystonia-parkinsonism in a second family. Neurology. 1997; 48(4):1066–1069. [PubMed: 9109901]
14. Dobyns WB, Ozelius LJ, Kramer PL, et al. Rapid-onset dystonia-parkinsonism. Neurology. 1993; 43(12):2596–2602. [PubMed: 8255463]
15. Kramer PL, Mineta M, Klein C, et al. Rapid-onset dystonia-parkinsonism: linkage to chromosome 19q13. Ann Neurol. 1999; 46(2):176–182. [PubMed: 10443882]
16. Pittock SJ, Joyce C, O'Keane V, et al. Rapid-onset dystonia-parkinsonism: a clinical and genetic analysis of a new kindred. Neurology. 2000; 55(7):991–995. [PubMed: 11061257]
17. Zaremba J, Mierzewska H, Lysiak Z, Kramer P, Ozelius LJ, Brashear A. Rapid-onset dystonia-parkinsonism: a fourth family consistent with linkage to chromosome 19q13. Mov Disord. 2004; 19(12):1506–1510. [PubMed: 15390049]
18. de Carvalho Aguiar P, Sweadner KJ, Penniston JT, et al. Mutations in the Na+/K+ -ATPase alpha3 gene ATP1A3 are associated with rapid-onset dystonia parkinsonism. Neuron. 2004; 43(2):169–175. [PubMed: 15260953]
19. Brashear A, Dobyns WB, de Carvalho Aguiar P, et al. The phenotypic spectrum of rapid-onset dystonia-parkinsonism (RDP) and mutations in the ATP1A3 gene. Brain. 2007; 130(Pt 3):828–835. [PubMed: 17282997]
20. McKeon A, Ozelius LJ, Hardiman O, Greenway MJ, Pittock SJ. Heterogeneity of presentation and outcome in the Irish rapid-onset dystonia-parkinsonism kindred. Mov Disord. 2007; 22(9):1325–1327. [PubMed: 17516473]
21. Barbano RL, Hill DF, Snively BM, et al. New triggers and non-motor findings in a family with rapid-onset dystonia-parkinsonism. Parkinsonism & related disorders. 2012; 18(6):737–741. [PubMed: 22534615]
22. Lee JY, Gollamudi S, Ozelius LJ, Kim JY, Jeon BS. ATP1A3 mutation in the first asian case of rapid-onset dystonia-parkinsonism. Mov Disord. 2007; 22(12):1808–1809. [PubMed: 17595045]
23. Zanotti-Fregonara P, Vidailhet M, Kas A, et al. [123I]-FP-CIT and [99mTc]-HMPAO single photon emission computed tomography in a new sporadic case of rapid-onset dystonia-parkinsonism. J Neurol Sci. 2008; 273(1–2):148–151. [PubMed: 18675996]
24. Blanco-Arias P, Einholm AP, Mamsa H, et al. A C-terminal mutation of ATP1A3 underscores the crucial role of sodium affinity in the pathophysiology of rapid-onset dystonia-parkinsonism. Hum Mol Genet. 2009; 18(13):2370–2377. [PubMed: 19351654]

25. Anselm IA, Sweadner KJ, Gollamudi S, Ozelius LJ, Darras BT. Rapid-onset dystonia-parkinsonism in a child with a novel *ATP1A3* gene mutation. Neurology. 2009; 73(5):400–401. [PubMed: 19652145]
26. Svetel M, Ozelius LJ, Buckley A, et al. Rapid-onset dystonia-parkinsonism: case report. J Neurol. 2010; 257(3):472–474. [PubMed: 19936820]
27. Kamm C, Fogel W, Wachter T, et al. Novel ATP1A3 mutation in a sporadic RDP patient with minimal benefit from deep brain stimulation. Neurology. 2008; 70(16 Pt 2):1501–1503. [PubMed: 18413579]
28. Tarsy D, Sweadner KJ, Song PC. Case records of the Massachusetts General Hospital. Case 17–2010 - a 29-year-old woman with flexion of the left hand and foot and difficulty speaking. N Engl J Med. 2010; 362(23):2213–2219. [PubMed: 20558373]
29. Roubergue A, Roze E, Vuillaumier-Barrot S, et al. The multiple faces of the ATP1A3-related dystonic movement disorder. Mov Disord. 2013; 28(10):1457–1459. [PubMed: 23483595]
30. Brashear A, Mink JW, Hill DF, et al. ATP1A3 mutations in infants: a new rapid-onset dystonia-Parkinsonism phenotype characterized by motor delay and ataxia. Dev Med Child Neurol. 2012; 54(11):1065–1067. [PubMed: 22924536]
31. Kamphuis DJ, Koelman H, Lees AJ, Tijssen MA. Sporadic rapid-onset dystonia-parkinsonism presenting as Parkinson's disease. Mov Disord. 2006; 21(1):118–119. [PubMed: 16161139]
32. Brashear A, Hill DF, B S, Sweadner KJ, Ozelius L. DeNovo and Recurrent Mutations in ATP1A3 Are Common in Rapid-Onset Dystonia-Parkinsonism. Neurology. 2010; 74(suppl 2):A204.
33. Heinzen EL, Swoboda KJ, Hitomi Y, et al. De novo mutations in ATP1A3 cause alternating hemiplegia of childhood. Nat Genet. 2012; 44(9):1030–1034. [PubMed: 22842232]
34. Rosewich H, Thiele H, Ohlenbusch A, et al. Heterozygous de-novo mutations in ATP1A3 in patients with alternating hemiplegia of childhood: a whole-exome sequencing gene-identification study. Lancet Neurol. 2012; 11(9):764–773. [PubMed: 22850527]
35. Ishii A, Saito Y, Mitsui J, et al. Identification of ATP1A3 mutations by exome sequencing as the cause of alternating hemiplegia of childhood in Japanese patients. PLoS One. 2013; 8(2):e56120. [PubMed: 23409136]
36. Petrovski S, Wang Q, Heinzen EL, Allen AS, Goldstein DB. Genic intolerance to functional variation and the interpretation of personal genomes. PLoS Genet. 2013; 9(8):e1003709. [PubMed: 23990802]
37. Brashear A, Farlow MR, Butler IJ, Kasarskis EJ, Dobyns WB. Variable phenotype of rapid-onset dystonia-parkinsonism. Mov Disord. 1996; 11(2):151–156. [PubMed: 8684384]
38. Brashear A, Butler IJ, Ozelius LJ, et al. Rapid-onset dystonia-parkinsonism: a report of clinical, biochemical, and genetic studies in two families. Adv Neurol. 1998; 78:335–339. [PubMed: 9750930]
39. Brashear A, Butler IJ, Hyland K, Farlow MR, Dobyns WB. Cerebrospinal fluid homovanillic acid levels in rapid-onset dystonia-parkinsonism. Ann Neurol. 1998; 43(4):521–526. [PubMed: 9546335]
40. Brashear A, Cook JF, Hill DF, et al. Psychiatric disorders in rapid-onset dystonia-parkinsonism. Neurology. 2012; 79(11):1168–1173. [PubMed: 22933743]
41. Bourgeois M, Aicardi J, Goutieres F. Alternating hemiplegia of childhood. J Pediatr. 1993; 122(5 Pt 1):673–679. [PubMed: 8496742]
42. Neville BG, Ninan M. The treatment and management of alternating hemiplegia of childhood. Dev Med Child Neurol. 2007; 49(10):777–780. [PubMed: 17880649]
43. Sweney MT, Silver K, Gerard-Blanluet M, et al. Alternating hemiplegia of childhood: early characteristics and evolution of a neurodevelopmental syndrome. Pediatrics. 2009; 123(3):e534–e541. [PubMed: 19254988]
44. Silver K, Andermann F. Alternating hemiplegia of childhood: a study of 10 patients and results of flunarizine treatment. Neurology. 1993; 43(1):36–41. [PubMed: 8423908]
45. Casaer P. Flunarizine in alternating hemiplegia in childhood. An international study in 12 children. Neuropediatrics. 1987; 18(4):191–195. [PubMed: 3320807]
46. Casaer P, Azou M. Flunarizine in alternating hemiplegia in childhood. Lancet. 1984; 2(8402):579. [PubMed: 6147627]

47. Sasaki M, Sakuragawa N, Osawa M. Long-term effect of flunarizine on patients with alternating hemiplegia of childhood in Japan. Brain Dev. 2001; 23(5):303–305. [PubMed: 11504600]
48. Kanavakis E, Xaidara A, Papathanasiou-Klontza D, Papadimitriou A, Velentza S, Youroukos S. Alternating hemiplegia of childhood: a syndrome inherited with an autosomal dominant trait. Dev Med Child Neurol. 2003; 45(12):833–836. [PubMed: 14667076]
49. Bassi MT, Bresolin N, Tonelli A, et al. A novel mutation in the ATP1A2 gene causes alternating hemiplegia of childhood. J Med Genet. 2004; 41(8):621–628. [PubMed: 15286158]
50. Swoboda KJ, Kanavakis E, Xaidara A, et al. Alternating hemiplegia of childhood or familial hemiplegic migraine? A novel ATP1A2 mutation. Ann Neurol. 2004; 55(6):884–887. [PubMed: 15174025]
51. Mikati MA, Maguire H, Barlow CF, et al. A syndrome of autosomal dominant alternating hemiplegia: clinical presentation mimicking intractable epilepsy; chromosomal studies; and physiologic investigations. Neurology. 1992; 42(12):2251–2257. [PubMed: 1361034]
52. Andermann E, Andermann F, Silver K, Levin S, Arnold D. Benign familial nocturnal alternating hemiplegia of childhood. Neurology. 1994; 44(10):1812–1814. [PubMed: 7936227]
53. Wagener-Schimmel LJ, Nicolai J. Child neurology: benign nocturnal alternating hemiplegia of childhood. Neurology. 2012; 79(18):e161–e163. [PubMed: 23109658]
54. Mikati MA, Kramer U, Zupanc ML, Shanahan RJ. Alternating hemiplegia of childhood: clinical manifestations and long-term outcome. Pediatr Neurol. 2000; 23(2):134–141. [PubMed: 11020638]
55. Saito Y, Sakuragawa N, Sasaki M, Sugai K, Hashimoto T. A case of alternating hemiplegia of childhood with cerebellar atrophy. Pediatr Neurol. 1998; 19(1):65–68. [PubMed: 9682890]
56. Saito Y, Inui T, Sakakibara T, Sugai K, Sakuma H, Sasaki M. Evolution of hemiplegic attacks and epileptic seizures in alternating hemiplegia of childhood. Epilepsy Res. 2010; 90(3):248–258. [PubMed: 20580529]
57. Hoei-Hansen CE, Dali CI, Lyngbye TJ, Duno M, Uldall P. Alternating hemiplegia of childhood in Denmark: Clinical manifestations and ATP1A3 mutation status. Eur J Paediatr Neurol. 2013
58. Sasaki M, Ishii A, Saito Y, et al. Genotype–Phenotype Correlations in Alternating Hemiplegia of Childhood. Neurology. 2014 (in press).
59. Dangond F, Garada B, Murawski BJ, Rey-Casserly C, Holman BL, Mikati MA. Focal brain dysfunction in a 41-year old man with familial alternating hemiplegia. Eur Arch Psychiatry Clin Neurosci. 1997; 247(1):35–41. [PubMed: 9088804]
60. Mikati M. Alternating hemiplegia of childhood. Pediatr Neurol. 1999; 21(4):764. [PubMed: 10580894]
61. Panagiotakaki E, Gobbi G, Neville B, et al. Evidence of a non-progressive course of alternating hemiplegia of childhood: study of a large cohort of children and adults. Brain. 2010; 133(Pt 12): 3598–3610. [PubMed: 20974617]
62. Di Michele M, Goubau C, Waelkens E, et al. Functional studies and proteomics in platelets and fibroblasts reveal a lysosomal defect with increased cathepsin-dependent apoptosis in ATP1A3 defective alternating hemiplegia of childhood. J Proteomics. 2013; 86:53–69. [PubMed: 23681173]
63. Post RL, Hegyvary C, Kume S. Activation by adenosine triphosphate in the phosphorylation kinetics of sodium and potassium ion transport adenosine triphosphatase. J Biol Chem. 1972; 247(20):6530–6540. [PubMed: 4263199]
64. Koenderink JB, Geibel S, Grabsch E, De Pont JJ, Bamberg E, Friedrich T. Electrophysiological analysis of the mutated Na, K-ATPase cation binding pocket. J Biol Chem. 2003; 278(51):51213–51222. [PubMed: 14532287]
65. Poulsen H, Khandelia H, Morth JP, et al. Neurological disease mutations compromise a C-terminal ion pathway in the Na(+)/K(+)-ATPase. Nature. 2010; 467(7311):99–102. [PubMed: 20720542]
66. Toustrup-Jensen M, Vilsen B. Importance of Glu(282) in transmembrane segment M3 of the Na(+), K(+)-ATPase for control of cation interaction and conformational changes. J Biol Chem. 2002; 277(41):38607–38617. [PubMed: 12149251]

67. Rodacker V, Toustrup-Jensen M, Vilsen B. Mutations Phe785Leu and Thr618Met in Na+, K+-ATPase, associated with familial rapid-onset dystonia parkinsonism, interfere with Na+ interaction by distinct mechanisms. J Biol Chem. 2006; 281(27):18539–18548. [PubMed: 16632466]
68. Einholm AP, Toustrup-Jensen MS, Holm R, Andersen JP, Vilsen B. The rapid-onset dystonia parkinsonism mutation D923N of the Na+, K+-ATPase alpha3 isoform disrupts Na+ interaction at the third Na+ site. J Biol Chem. 2010; 285(34):26245–26254. [PubMed: 20576601]
69. Toustrup-Jensen MS, Einholm AP, Schack VR, et al. Relationship between intracellular Na+ concentration and reduced Na+ affinity in Na+, K+-ATPase mutants causing neurological disease. J Biol Chem. 2013
70. Ogawa H, Shinoda T, Cornelius F, Toyoshima C. Crystal structure of the sodium-potassium pump (Na+, K+-ATPase) with bound potassium and ouabain. Proc Natl Acad Sci U S A. 2009; 106(33): 13742–13747. [PubMed: 19666591]
71. Kirshenbaum GS, Dawson N, Mullins JGL, et al. Alternating Hemiplegia of Childhood-Related Neural and Behavioural Phenotypes in Na+, K+-ATPase α3 Missense Mutant Mice. PLoS ONE. 2013; 8(3):e60141. [PubMed: 23527305]
72. Kaneko M, Desai BS, Cook B. Ionic leakage underlies a gain-of-function effect of dominant disease mutations affecting diverse P-type ATPases. Nat Genet. 2013
73. Clapcote SJ, Duffy S, Xie G, et al. Mutation I810N in the alpha3 isoform of Na+, K+-ATPase causes impairments in the sodium pump and hyperexcitability in the CNS. Proc Natl Acad Sci U S A. 2009; 106(33):14085–14090. [PubMed: 19666602]
74. Heinzen EL, Swoboda KJ, Hitomi Y, et al. De novo mutations in ATP1A3 cause alternating hemiplegia of childhood. Nat Genet. 2012
75. Kirshenbaum GS, Clapcote SJ, Duffy S, et al. Mania-like behavior induced by genetic dysfunction of the neuron-specific Na+, K+-ATPase alpha3 sodium pump. Proc Natl Acad Sci U S A. 2011; 108(44):18144–18149. [PubMed: 22025725]
76. Kirshenbaum GS, Saltzman K, Rose B, Petersen J, Vilsen B, Roder JC. Decreased neuronal Na+, K+ - ATPase activity in Atp1a3 heterozygous mice increases susceptibility to depression-like endophenotypes by chronic variable stress. Genes Brain Behav. 2011; 10(5):542–550. [PubMed: 21418141]
77. Ferrari P, Ferrandi M, Valentini G, Bianchi G. Rostafuroxin: an ouabain antagonist that corrects renal and vascular Na+-K+- ATPase alterations in ouabain and adducin-dependent hypertension. Am J Physiol Regul Integr Comp Physiol. 2006; 290(3):R529–R535. [PubMed: 16467500]
78. Moseley AE, Williams MT, Schaefer TL, et al. Deficiency in Na, K-ATPase alpha isoform genes alters spatial learning, motor activity, and anxiety in mice. J Neurosci. 2007; 27(3):616–626. [PubMed: 17234593]
79. DeAndrade MP, Yokoi F, van Groen T, Lingrel JB, Li Y. Characterization of Atp1a3 mutant mice as a model of rapid-onset dystonia with parkinsonism. Behav Brain Res. 2011; 216(2):659–665. [PubMed: 20850480]
80. Ikeda K, Satake S, Onaka T, et al. Enhanced inhibitory neurotransmission in the cerebellar cortex of Atp1a3-deficient heterozygous mice. J Physiol. 2013; 591(Pt 13):3433–3449. [PubMed: 23652595]
81. Pizoli CE, Jinnah HA, Billingsley ML, Hess EJ. Abnormal cerebellar signaling induces dystonia in mice. J Neurosci. 2002; 22(17):7825–7833. [PubMed: 12196606]
82. Calderon DP, Fremont R, Kraenzlin F, Khodakhah K. The neural substrates of rapid-onset Dystonia-Parkinsonism. Nat Neurosci. 2011; 14(3):357–365. [PubMed: 21297628]
83. Sweadner KJ. Isozymes of the Na+/K+-ATPase. Biochim Biophys Acta. 1989; 988(2):185–220. [PubMed: 2541792]
84. Rajarao SJ, Canfield VA, Mohideen MA, et al. The repertoire of Na, K-ATPase alpha and beta subunit genes expressed in the zebrafish, Danio rerio. Genome Res. 2001; 11(7):1211–1220. [PubMed: 11435403]
85. Doganli C, Beck HC, Ribera AB, Oxvig C, Lykke-Hartmann K. alpha3Na+/K+-ATPase deficiency causes brain ventricle dilation and abrupt embryonic motility in zebrafish. J Biol Chem. 2013; 288(13):8862–8874. [PubMed: 23400780]

86. Lebovitz RM, Takeyasu K, Fambrough DM. Molecular characterization and expression of the (Na + + K+)-ATPase alpha-subunit in Drosophila melanogaster. EMBO J. 1989; 8(1):193–202. [PubMed: 2540956]

87. Palladino MJ, Bower JE, Kreber R, Ganetzky B. Neural dysfunction and neurodegeneration in Drosophila Na+/K+ ATPase alpha subunit mutants. J Neurosci. 2003; 23(4):1276–1286. [PubMed: 12598616]

88. Ashmore LJ, Hrizo SL, Paul SM, Van Voorhies WA, Beitel GJ, Palladino MJ. Novel mutations affecting the Na, K ATPase alpha model complex neurological diseases and implicate the sodium pump in increased longevity. Hum Genet. 2009; 126(3):431–447. [PubMed: 19455355]

89. Kirshenbaum GS, Dawson N, Mullins JG, et al. Alternating hemiplegia of childhood-related neural and behavioural phenotypes in Na+, K+-ATPase alpha3 missense mutant mice. PLoS One. 2013; 8(3):e60141. [PubMed: 23527305]

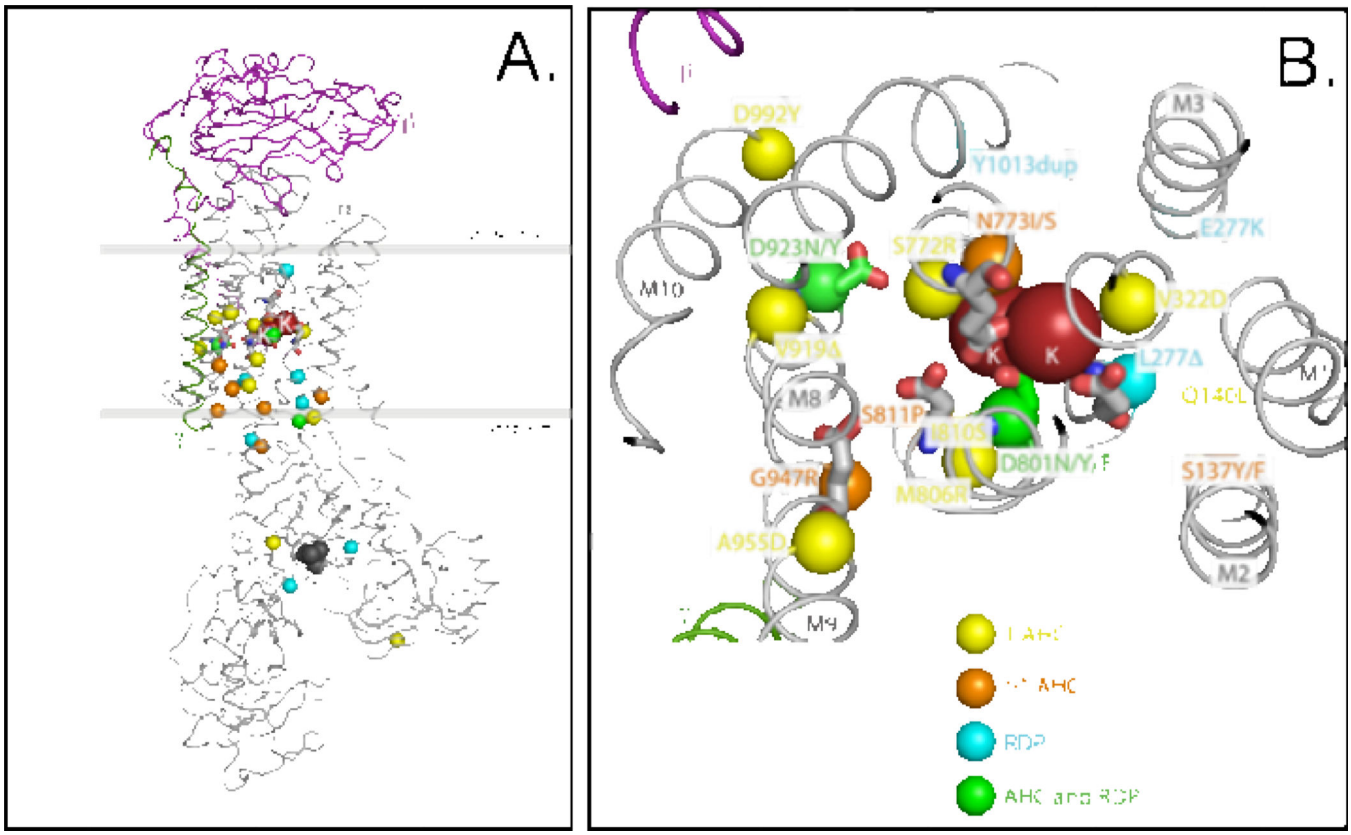

Figure 1.

Structure of the Na, K-ATPase. **A.** Cartoon representation of the Na, K-ATPase in the potassium occluded state showing K^+ (red spheres), the three protein subunits α (grey), β(purple) and FXYDγ (green), and the phosphorylation, which is mimicked by MgF_4^{2-} (dark gray). Residues reported to be mutated in AHC and/or RDP (cf. Table 1) are indicated by spheres at the α carbon, yellow for one case of AHC, orange for more than one case of AHC, cyan for RDP, green for both AHC and RDP. Key ion binding residues are shown in stick. **B.** A 90° degree rotation in the membrane plane of the representation in A giving an extracellular view of the ion binding transmembrane part of the Na, K-ATPase with disease-causing mutations color coded as in A. Two of the ion binding residues (shown in sticks) have been found to be mutated both in RDP and in AHC patients. The figures were made from pdb code 2ZXE.

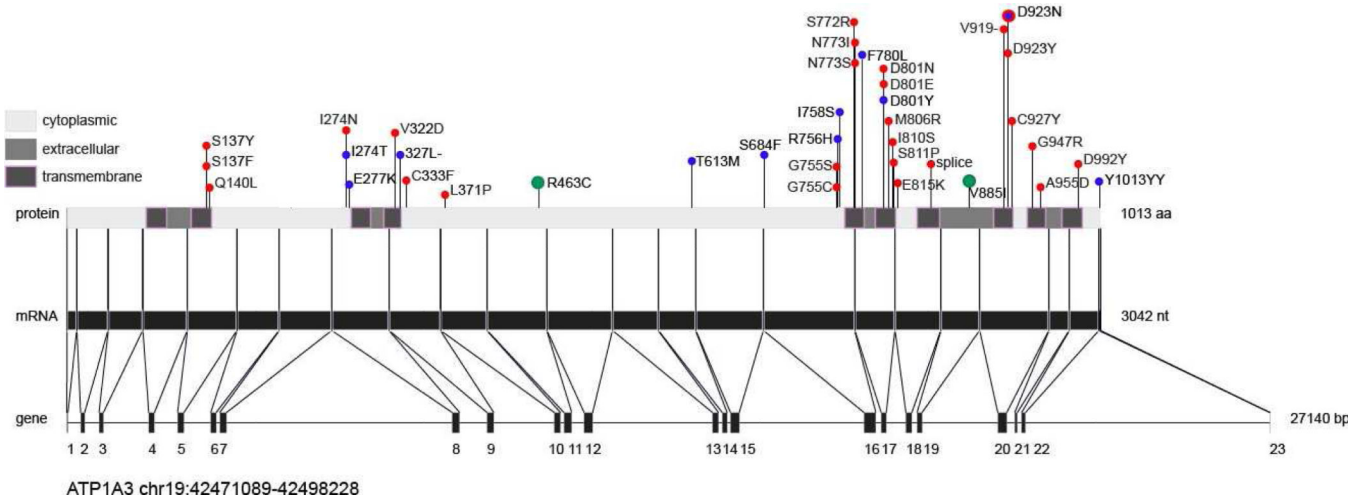

Figure 2.

Schematic depicting the location of AHC-causing (red dots) and RDP-causing (blue dots) mutations in *ATP1A3*, mRNA and protein. The one mutation shared between disease phenotypes is located at D923N (blue dot with a red dot inside). Two rare polymorphisms identified in the general population are indicated by the green dots. Amino acid modifications are provided to the right of the dots.

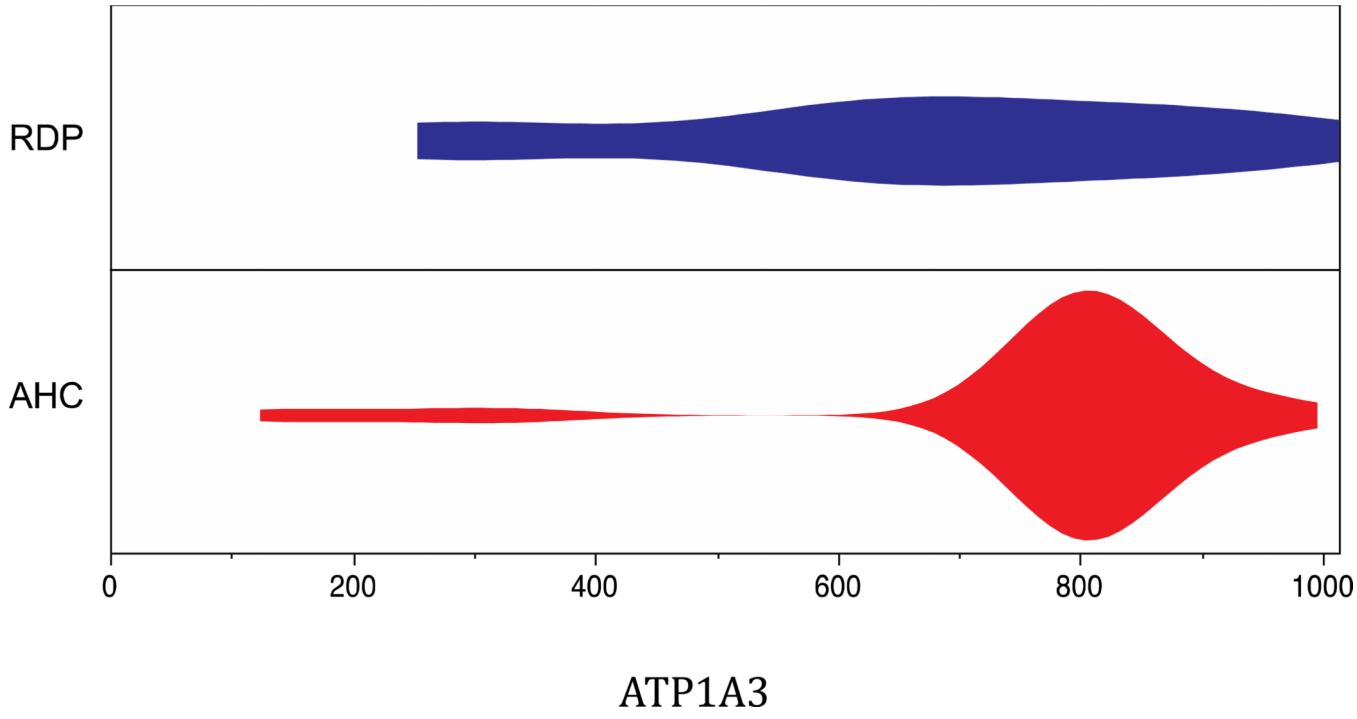

Figure 3.

Density plot showing the distribution of AHC and RDP mutations identified to date in 116 and 20 patients with *ATP1A3* mutations, respectively. In general, RDP mutations appear to be more evenly distributed, whereas AHC mutations are heavily concentrated in particular sites in the protein.

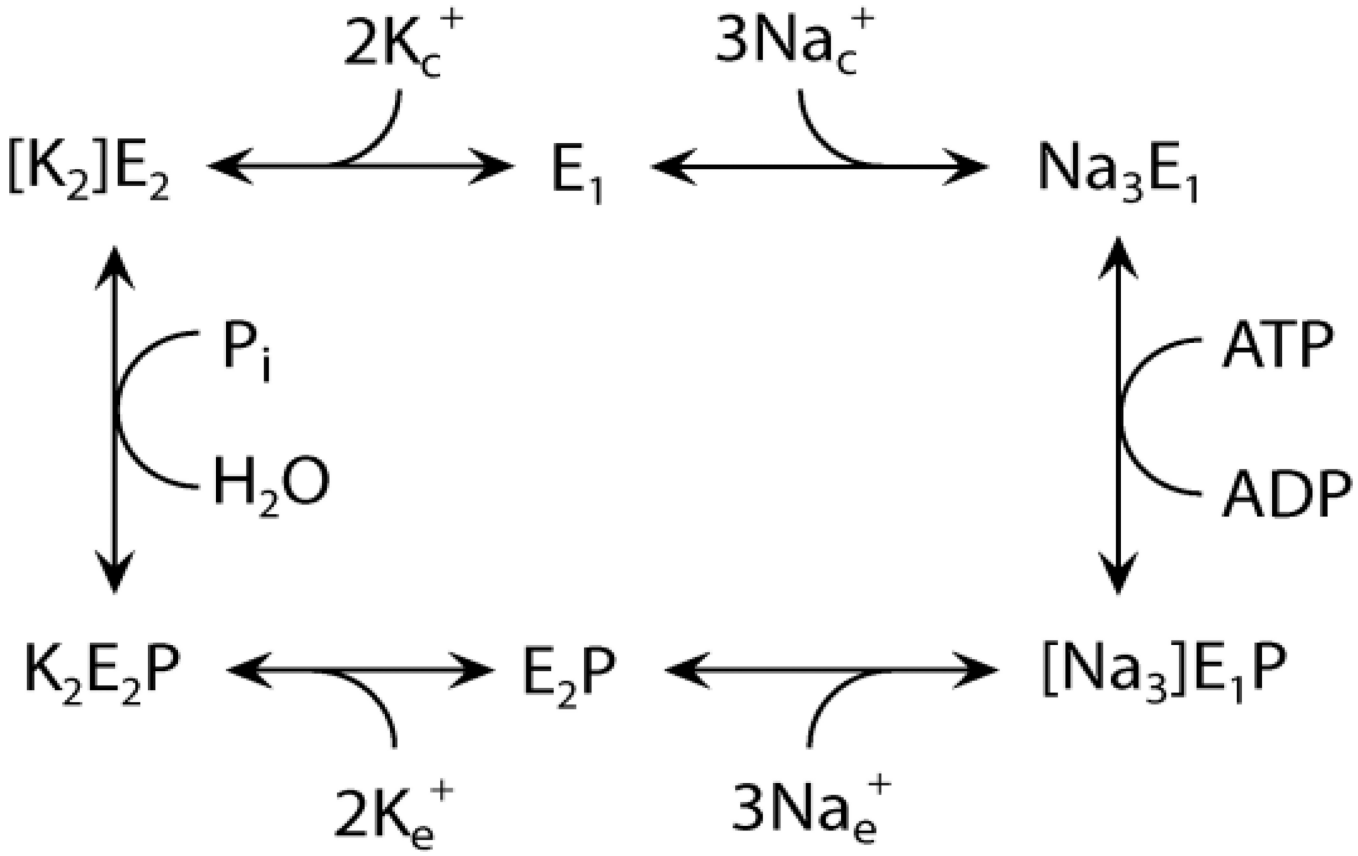

Figure 4.

Post-Albers model[63] for the Na, K-ATPase reaction cycle, as reproduced from Toustrup-Jensen and co-workers.[69]. E1 and E2 are major conformational states with preference for binding of Na^+ and K^+, respectively. Cytoplasmic and extracellular ions are indicated by subscripts c and e, respectively. Brackets indicate occlusion of the ions in a cavity in the protein. P indicates the bound phosphate.

A ***Atp1a3* mutant mice**

	Genetic lesion	NKA α3 abundance	Brain NKA activity	Homozygote phenotype	Heterozygote phenotype: Visible defects	Heterozygote phenotype: Open field activity	Heterozygote phenotype: Balance beam performance	Heterozygote phenotype: Response to stress
Myshkin	I810N	=	↓ (40%)	Perinatal death	Tremor Ataxia Seizures Small body	↑	↓	Seizures
Atp1a3^{tm1Ling}	Point mutation in intron 4	↓ (60%)	↓ (16%)	Perinatal death	None	↑	=	Mild balance deficit (♀ only)
Atp1a3^{tm2Kwk}	Deletion of exons 2-6	n.d.	n.d.	Perinatal death	None	↑	↑	=

B ***Atpα* mutant *Drosophila***

Allele name	Genetic lesion	AHC α3 equivalent	NKA α abundance	Heterozygote phenotype: Locomotor activity	Heterozygote phenotype: Temperature stress response	Heterozygote phenotype: Mechanical stress response	Heterozygote phenotype: Neuropathology
Atpα [CJ10]	G744S	G755S	=	↓	Paralysis	Paralysis	Vacuolar

C ***Atp1a3a/b* knockdown zebrafish**

Knockdown	α3 transcript abundance	60 hpf embryo phenotype: Brain ventricles	60 hpf embryo phenotype: Spontaneous motility	60 hpf embryo phenotype: Touch response
Atp1a3a	↓ (62%)	Dilation	Abnormal	↓
Atp1a3b	↓ (66%)	Dilation	Abnormal	↓

Figure 5.

Na, K-ATPase α3 genetic animal models. **A.** *Atp1a3* mutant mice. The locations of three mutations in the mouse *Atp1a3* genomic locus are depicted. *Myshkin* mice carry a T-to-A transversion in exon 18 that results in the substitution of asparagine for isoleucine at position 810 (I810N)[73,89] *Atp1a3*^{tm1Ling} mice carry a point mutation in intron 4 adjacent to the exon-intron splice site that results in aberrant splicing of the gene, adding 126 base pairs to the RNA transcript[73,78,79]. *Atp1a3*^{tm2Kwk} mice carry a STOP-polyA cassette that replaces exons 2–6 in *Atp1a3*[80]. **B.** *Atp*α mutant *Drosophila*. *Atp*α^{CJ10} fruit flies carry a G to A transition that results in the substitution of glycine for serine at position 744 (G744S)[88]. G744S in the *Drosophila* α subunit is equivalent to mutation G755S in the human α3 subunit found in an AHC patient[33]. **C.** *Atp1a3a*/*b* knockdown zebrafish. Knockdown of *Atp1a3a* or *Atp1a3b* RNA transcript by ~65% in 60 hpf embryos had similar phenotypic effects[85].

Table 1

Disease-causing *ATP1A3* mutations

□ *ATP1A3* mutation coordinates are defined based on UniProt ID P1363724 and Consensus CDS ID CCDS12594.1.

***ATP1A3* mutation**	**Disease/population**	**Disease inheritance**	**Number of unrelated patients with the mutation§**
c.410C>T;p.S137F	AHC	Sporadic	1
c.410C>A;p.S137Y	AHC	Sporadic	2
c.419A>T;p.Q140L	AHC	Sporadic	1
c.821T>A;p.I274N	AHC	Sporadic, familial	2
c.821T>C;p.I274T	RDP	Sporadic	1
c.829G>A;p.E277K	RDP	Sporadic	2
c.965T>A;p.V322D	AHC	Sporadic	1
c.979_981delCTG;p.327Ldel	RDP	Sporadic	1
c.998G>T;p.C333F	AHC	Sporadic	2
c.1112T>C;p.L371P	AHC	Sporadic	1
c.1387C>T;R463C	rare polymorphic variant in the general population	not applicable	not applicable
c.1838C>T;p.T613M	RDP	Sporadic, familial	
c.2051C>T;p.S684F	RDP	Sporadic	1
c.2263G>T;p.G755C	AHC	Sporadic	2
c.2263G>A;p.G755S	AHC	Sporadic	1
c.2267G>A;p.R756H	RDP	Sporadic	1
c.2273T>G;p.I758S	RDP	Familial	1
c.2316C>A;p.S772R	AHC	Sporadic	1
c.2318A>T;p.N773I	AHC	Sporadic	1
c.2318A>G;p.N773S	AHC	Sporadic	1
c.2338T>C;p.F780L	RDP	Familial	1
c.2401G>A;p.D801N	AHC	Sporadic	49
c.2401G>T;p.D801Y	RDP	Familial	1
c.2403T>A;p.D801E	AHC	Sporadic	1
c.2417T>G;p.M806R	AHC	Sporadic	1
c.2429T>G;p.I810S	AHC	Sporadic	1
c.2431T>C;p.S811P	AHC	Sporadic	4
c.2443G>A;p.E815K	AHC	Sporadic	31
c.2542+1G>A;splice site	AHC	Sporadic	2
c.2653G>A;V885I	rare polymorphic variant in the general population	Not applicable	not applicable
c.2755_2757delGTC;p.V919del	AHC	Sporadic	1
c.2767G>A;p.D923N	RDP, AHC	Sporadicand familial RDP, familial AHC	RDP Sporadic: 2 RDP familial: 2 AHC familial: 1

***ATP1A3* mutation**	**Disease/population**	**Disease inheritance**	**Number of unrelated patients with the mutation[§]**
c.2767G>T;p.D923Y	AHC	Sporadic	1
c.2780G>A;p.C927Y	AHC	Sporadic	1
c.2839G>A;p.G947R	AHC	Sporadic	5
c.2839G>C;p.G947R	AHC	Sporadic	2
c.2864C>A;p.A955D	AHC	Sporadic	1
c.2974G>T;p.D992Y	AHC	Sporadic	1
c.3038_3040dupACT;p.1013Ydup	RDP	Sporadic	1

[§]compiled from refs[18–30,33–35,57]

[□]Mutation c.658G>A;p.D220N previously reported as causal in Heinzen and co-workers[33] was later shown to be a rare, inherited mutation, and that the disease-causing mutation in this patient is a previously overlooked *de novo* D801N *ATP1A3* mutation (unpublished data). As such D220N has been removed from this table and one additional patient has been counted as having a D801N mutation.

Table 2

Summary of *Drosophila* Na, K-ATPase α subunit mutant phenotypes

Fly α mutation	Human α3 equivalent	TS paralysis	MS paralysis	Body weight	Protein abundance
S201L	S212	No	Yes	↓	=
P262L	P273	No	Yes	=	=
S348T	S359	No	Yes	=	↓
G528S	G539	No	Yes	=	=
A588T	A599	No	Yes	=	↓
G744S	$G755^{AHC}$	Yes	Yes	=	=
D981N	$D992^{AHC}$	Yes	Mild	n.d.	=
E982K	D993	Yes	Mild	n.d.	=

TS, temperature-sensitive; MS, mechanical stress-induced; AHC, equivalent amino acid residue that is substituted in AHC; Mild, when maintained at 28°C; =, no different than wild-type; ↓, lower than wild-type; n.d., not determined. Adapted from Ashmore et al.[88]