

Minerva Access is the Institutional Repository of The University of Melbourne

Author/s:

Viollet, L;Glusman, G;Murphy, KJ;Newcomb, TM;Reyna, SP;Sweney, M;Nelson, B;Andermann, F;Andermann, E;Acsadi, G;Barbano, RL;Brown, C;Brunkow, ME;Chugani, HT;Cheyette, SR;Collins, A;DeBrosse, SD;Galas, D;Friedman, J;Hood, L;Huff, C;Jorde, LB;King, MD;LaSalle, B;Leventer, RJ;Lewelt, AJ;Massart, MB;Mérida, MR;Ptáček, LJ;Roach, JC;Rust, RS;Renault, F;Sanger, TD;De Menezes, MAS;Tennyson, R;Uldall, P;Zhang, Y;Zupanc, M;Xin, W;Silver, K;Swoboda, KJ

Title:

Alternating hemiplegia of childhood: Retrospective genetic study and genotype-phenotype correlations in 187 subjects from the US AHCF registry

Date:

2015-05-21

Citation:

Viollet, L., Glusman, G., Murphy, K. J., Newcomb, T. M., Reyna, S. P., Sweney, M., Nelson, B., Andermann, F., Andermann, E., Acsadi, G., Barbano, R. L., Brown, C., Brunkow, M. E., Chugani, H. T., Cheyette, S. R., Collins, A., DeBrosse, S. D., Galas, D., Friedman, J., ... Swoboda, K. J. (2015). Alternating hemiplegia of childhood: Retrospective genetic study and genotype-phenotype correlations in 187 subjects from the US AHCF registry. *Plos One*, 10 (5), <https://doi.org/10.1371/journal.pone.0127045>.

Persistent Link:

<https://hdl.handle.net/11343/261181>

License:

CC BY

RESEARCH ARTICLE

Alternating Hemiplegia of Childhood: Retrospective Genetic Study and Genotype-Phenotype Correlations in 187 Subjects from the US AHCF Registry

Louis Viollet¹, Gustavo Glusman², Kelley J. Murphy¹, Tara M. Newcomb¹, Sandra P. Reyna¹, Matthew Sweney¹, Benjamin Nelson¹, Frederick Andermann³, Eva Andermann³, Gyula Acsadi⁴, Richard L. Barbano⁵, Candida Brown⁶, Mary E. Brunkow², Harry T. Chugani⁷, Sarah R. Cheyette⁸, Abigail Collins⁹, Suzanne D. DeBrosse¹⁰, David Galas¹¹, Jennifer Friedman¹², Lee Hood², Chad Huff¹³, Lynn B. Jorde¹⁴, Mary D. King¹⁵, Bernie LaSalle¹⁶, Richard J. Leventer¹⁷, Aga J. Lewelt¹⁸, Mylynda B. Massart¹⁹, Mario R. Mérida, II²⁰, Louis J. Ptáček²¹, Jared C. Roach², Robert S. Rust²², Francis Renault²³, Terry D. Sanger²⁴, Marcio A. Sotero de Menezes²⁵, Rachel Tennyson¹, Peter Uldall²⁶, Yue Zhang²⁷, Mary Zupanc²⁸, Winnie Xin²⁹, Kenneth Silver³⁰, Kathryn J. Swoboda^{1*}



OPEN ACCESS

Citation: Viollet L, Glusman G, Murphy KJ, Newcomb TM, Reyna SP, Sweney M, et al. (2015) Alternating Hemiplegia of Childhood: Retrospective Genetic Study and Genotype-Phenotype Correlations in 187 Subjects from the US AHCF Registry. PLoS ONE 10(5): e0127045. doi:10.1371/journal.pone.0127045

Academic Editor: Fengfeng Zhou, Shenzhen Institutes of Advanced Technology, CHINA

Received: December 12, 2014

Accepted: April 11, 2015

Published: May 21, 2015

Copyright: © 2015 Viollet et al. This is an open access article distributed under the terms of the [Creative Commons Attribution License](http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Data Availability Statement: All relevant data are within the paper and its Supporting Information files.

Funding: The Alternating Hemiplegia of Childhood Foundation (<http://ahckids.org/>). Funds were received by KJS. The association Genetique Actions (<http://www.genetique-actions.fr/>). Funds were received by LV. The University of Luxembourg—Institute for Systems Biology Strategic Partnership (<http://www.partnerships.systemsbio.net/517/>). Funds were received by LH. The Luxembourg Centre for Systems Biomedicine (<http://www.lcsb.uni.lu/>). Funds were

1 Pediatric Motor Disorders Research Program, Departments of Neurology and Pediatrics, University of Utah, Salt Lake City, Utah, United States of America, **2** Institute for Systems Biology, Seattle, Washington, United States of America, **3** Neurogenetics Unit, Montreal Neurologic Institute and Hospital, McGill University, Montreal Quebec, Canada, **4** Departments of Pediatrics and Neurology, Connecticut Children's Medical Center and University of Connecticut School of Medicine, Hartford, CT, United States of America, **5** Department of Neurology, University of Rochester School of Medicine, Rochester, New York, United States of America, **6** Diablo Valley Child Neurology, an affiliate of Stanford Health Alliance, Pleasant Hill, California, United States of America, **7** Division of Pediatric Neurology, Children's Hospital of Michigan, Wayne State University, Detroit, Michigan, United States of America, **8** Department of Child Neurology, Palo Alto Medical Foundation Redwood City Clinic, Redwood City, California, United States of America, **9** Department of Pediatric Neurology, Children's Hospital Colorado, University of Colorado Hospital, Aurora, Colorado, United States of America, **10** Departments of Genetics and Genome Sciences, Pediatrics, and Neurology, Case Western Reserve University and University Hospitals Case Medical Center, Cleveland, Ohio, United States of America, **11** Pacific Northwest Diabetes Research Institute, Seattle, Washington, United States of America, **12** Departments of Neuroscience and Pediatrics, University of California San Diego, San Diego, California, United States of America, **13** Department of Epidemiology, The University of Texas M.D. Anderson Cancer Center, Houston, Texas, United States of America, **14** Department of Human Genetics, University of Utah, Salt Lake City, Utah, United States of America, **15** Departments of Pediatrics and Neurology, University College Dublin School of Medicine and Medical Science, Dublin, Ireland, **16** Department of Biomedical Informatics, University of Utah School of Medicine, Salt Lake City, Utah, United States of America, **17** Children's Neuroscience Centre, Murdoch Childrens Research Institute, University of Melbourne Department of Paediatrics, The Royal Children's Hospital Melbourne, Parkville Victoria, Australia, **18** Department of Pediatrics, College of Medicine Jacksonville, University of Florida, Jacksonville, Florida, United States of America, **19** Department of Family Medicine, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania, United States of America, **20** Stevens Henager College, Salt Lake City, Utah, United States of America, **21** Department of Neurology, University of California San Francisco, San Francisco, California, United States of America, **22** Center for Medical Ethics and Humanities in Medicine, University Of Virginia UVA health system, Charlottesville, Virginia, United States of America, **23** Departement de Neurophysiologie. Hopital Armand Trousseau APHP, Paris, France, **24** Department of Biomedical Engineering, University of Southern California, Los Angeles, California, United States of America, **25** Swedish Neuroscience Institute, Swedish Medical Center, Seattle, Washington, United States of America, **26** Department of Paediatrics and Adolescent Medicine, Juliane Marie Centre, Rigshospitalet, University of Copenhagen, Copenhagen, Denmark, **27** Study Design and Biostatistics Center, University of Utah, Salt Lake City, Utah, United States of America, **28** Department of Neurology, Children's Hospital Orange County, and Department of Pediatrics, University of California, Orange, California, United States of America, **29** Center for Human Genetic Research, Department of Neurology, Massachusetts General Hospital, Boston, Massachusetts, United States of America, **30** Departments of Pediatrics and Neurology,

received by LH. The National Institute of General Medical Sciences Center for Systems Biology (<http://www.nigms.nih.gov/research/specificareas/sysbio/Pages/default.aspx>) P50 GM076547. Funds were received by LH. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

University of Chicago and Comer Children's Hospital, Chicago, Illinois, United States of America

* swoboda@genetics.utah.edu

Abstract

Mutations in *ATP1A3* cause Alternating Hemiplegia of Childhood (AHC) by disrupting function of the neuronal Na⁺/K⁺ ATPase. Published studies to date indicate 2 recurrent mutations, D801N and E815K, and a more severe phenotype in the E815K cohort. We performed mutation analysis and retrospective genotype-phenotype correlations in all eligible patients with AHC enrolled in the US AHC Foundation registry from 1997-2012. Clinical data were abstracted from standardized caregivers' questionnaires and medical records and confirmed by expert clinicians. We identified *ATP1A3* mutations by Sanger and whole genome sequencing, and compared phenotypes within and between 4 groups of subjects, those with D801N, E815K, other *ATP1A3* or no *ATP1A3* mutations. We identified heterozygous *ATP1A3* mutations in 154 of 187 (82%) AHC patients. Of 34 unique mutations, 31 (91%) are missense, and 16 (47%) had not been previously reported. Concordant with prior studies, more than 2/3 of all mutations are clustered in exons 17 and 18. Of 143 simplex occurrences, 58 had D801N (40%), 38 had E815K (26%) and 11 had G937R (8%) mutations. Patients with an E815K mutation demonstrate an earlier age of onset, more severe motor impairment and a higher prevalence of status epilepticus. This study further expands the number and spectrum of *ATP1A3* mutations associated with AHC and confirms a more deleterious effect of the E815K mutation on selected neurologic outcomes. However, the complexity of the disorder and the extensive phenotypic variability among subgroups merits caution and emphasizes the need for further studies.

Introduction

Alternating Hemiplegia of Childhood (AHC, MIM#614820) is a rare and complex neurodevelopmental disorder described initially by Verret and Steele in 1971, and named for the characteristic recurrent attacks of hemiplegia that affected first one side of the body, then another. However, episodes of paroxysmal neurologic dysfunction in this unusual disorder encompass a wide range of abnormal movements and episode types ranging from hemiplegia to quadriplegia to dystonia, and lasting from minutes to hours or even days. A variety of external or emotional stressors trigger the onset or worsening of paroxysmal symptoms, but they are consistently relieved by sleep, whether pharmacologically or naturally induced [1,2]. Although rare familial cases with autosomal dominant inheritance have been reported [3–6], AHC is predominantly a sporadic disorder. Estimated incidence of the classic form of the disease is 1 in one million: males and females are affected in roughly equal numbers. The variability and the complexity of presenting symptoms have historically led to considerable delays in diagnosis, which was until recently based solely on carefully conceived clinical diagnostic criteria. These criteria specified an infantile onset (< 18 months) of recurrent paroxysmal episodes of hemiplegia, dystonia and ocular movement abnormalities, and were ultimately characterized by the appearance of additional fixed neurologic signs and symptoms including chorea, dysarthria, dyskinesia, ocular apraxia, ataxia and global developmental delay [2,7,8]. The variable course

of the disease and limited longitudinal follow-up and neuropathologic data have led to ongoing speculation as to whether or not AHC represents a static neurodevelopmental or a progressive disorder [9–11].

The pathophysiology of AHC was totally unknown until the recent identification of mutations in *ATP1A3*, which encodes a neuron specific sodium/potassium ATPase involved in the regulation of neuronal excitability [12]. We and others initially identified de novo recurrent mutations in *ATP1A3* by performing whole exome studies in trios in a series of simplex AHC patients. These initial studies indicated that more than 2/3 of patients had de novo mutations, confirming a causative role of *ATP1A3* in the pathogenesis of AHC, and that two mutations accounted for most of the confirmed cases [13–21]. *ATP1A3* was previously shown to be mutated in Rapid-Onset Dystonia-Parkinsonism (RDP, DYT12, MIM#128235), a rare neurological disorder characterized by abrupt onset of dystonia and triggered by emotional or physical stress [22–37]. More recently, mutations were identified in another childhood onset condition called CAPOS syndrome (MIM#601338) with paroxysmal neurological symptoms overlapping with AHC and DYT12 but with distinctive features including pes cavus, optic atrophy and sensorineural hearing loss [38,39]. To date, mutations in *ATP1A3* for these different phenotypes have proved largely non-overlapping, indicating that genotypes may be predictive of phenotype in these allelic disorders, at least for patients presenting with the classic phenotypes as defined using strict clinical diagnostic criteria. Since *ATP1A3* mutations were reported in patients with AHC in 2012, the increasing numbers of patients with overlapping or unique phenotypes have led to use of the broader term “*ATP1A3*-related neurologic disorders.” However, it is notable that, to date, patients with the classically described phenotypes associated with AHC and RDP share very little overlap in the precise mutations identified. This underscores the importance of genotype-phenotype studies and that will undoubtedly provide clues to understand the unique pathogenesis of these clinical syndromes.

Here we present the results of a genetic and clinical study of the largest AHC cohort reported to date. This cohort includes 187 AHC patients enrolled since 1997 in the US AHC foundation (AHCF) registry and biobank at the University of Utah. We identified 34 unique mutations in *ATP1A3* among 154 affected individuals, accounting for 82% of cases (154/187) and confirming the previously observed high frequency of the D801N and E815K mutations among AHC patients. In a subset of patients with complete clinical data, we performed a retrospective genotype-phenotype correlation study to evaluate the influence of the two most frequently observed mutations on selected outcomes in patients with a clinical diagnosis of AHC.

Patients

One hundred eighty seven patients (82 males and 105 females) with AHC enrolled in the registry from 1997 to 2012 and provided DNA samples and clinical information for this analysis. Subjects were predominantly from the USA (108) but also from Australia (6), Brazil (2), Canada (7), Chile (1), Croatia (1), Czech Republic (2), Denmark (2), France (27), Iceland (1), Ireland (2), India (2), Israel (1), Italy (5), Kenya (1), Mexico (1), the Netherlands (1), New Zealand (1), Puerto Rico (4), Spain (4), Sweden (2), Turkey (1), and the United Kingdom (4). Clinical diagnosis of AHC had been confirmed in all cases by a neurologist based on published diagnostic criteria for AHC; a second review of available history and records was performed by authors KJS, KS or MS. Typical cases, defined as “clinically definite,” fulfilled the diagnostic criteria as originally defined by Bourgeois et al.² Atypical cases deemed “clinically probable” by the referring physician were also included in this analysis and included subjects with onset of first symptoms > 18 months of age but whose phenotype fulfilled all other diagnostic criteria for AHC.

One hundred sixty-nine patients were simplex cases, and DNAs of both parents were available for 70 (41%). The cohort included two sets of monozygotic twins who were concordantly affected. Fourteen subjects in 5 multiplex pedigrees, 2 with dominant inheritance, were also included in the present analysis. Patients and their parents gave informed written consent for clinical data and DNA storage and analysis. Ethics approval was obtained from the Institutional Review Board at the University of Utah. Among the 187 patients, 13 have been included in the genetic study published by Heinzen et al in 2012 [12].

Methods

DNA was extracted from peripheral venous blood using QIAmp DNA minikit (Qiagen, Hilden, Germany). *ATP1A3* sequencing was performed on DNA from AHC probands and family members using the Sanger method after PCR amplification of the 23 coding exons and their flanking regions with intronic primers as defined by Heinzen et al (reference sequence ENSG00000105409/NCBI:NM_001256214.1) [12]. PCR products were purified with ExoSA-P-IT (Affymetrix, Santa Clara, CA) and sequenced using the BigDye3.1 terminator method on an ABI PRISM 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA). Sequences were analyzed with Sequencher 4.9 Software (Genes Codes Corporation, Ann Harbor, MI) and DNA variants were described using ALAMUT Software (Interactive-Biosoftware, France). Evaluation of pathogenicity of the DNA variants was made using the Polymorphism Phenotyping (Polyphen) program (<http://genetics.bwh.harvard.edu/pph/>), the Provean program (<http://provean.jcvi.org/index.php>) and the Human Splicing Finder tool (<http://www.umd.be/HSF/>). Detection of the proband's mutation in the DNA of the parents was performed by Sanger sequencing of the mutated exon. DNA from an additional 30 probands was examined via whole genome sequencing on the CGI platform (Complete Genomics Inc., Mountain View, CA), including 25 parent-child trios and an additional 5 multiplex AHC pedigrees. Sequences were aligned to the GRCh37 (hg19) human reference genome. Variants were evaluated using QIAGEN's Ingenuity Variant Analysis software. We tested for presence and frequency of identified variants in the Kaviar database (version 141029), which integrates data from 1000 genomes, the Personal Genome Project, ESP6500, dbSNP and several other public resources, and thousands of private genome sequences [40].

For the clinical phenotype portion of the study, information was collected using standardized questionnaires completed by the parent or caregiver first, and then validated by the physician. For some patients, follow-up information was available from medical records, subsequent in-person evaluations at one of several national family meetings sponsored by the AHCF, or via follow-up telephone interviews. Main characteristics of the subset of AHC patients with complete clinical data are summarized in Table 1. Clinical characteristics were compared among a cohort of 164 subjects with and without *ATP1A3* mutations. We also compared clinical data between individual patient cohorts with the two most frequent mutations, D801N and E815K, and to those with other *ATP1A3* mutations.

Table 1. Summary of 164 AHC patients included in the genotype-phenotype correlation study.

<i>ATP1A3</i> mutations	E815K	D801N	Other Mutations	No Mutation
Total Number of Subjects	31	58	49	26
Females	16 (51.6%)	36 (62.1%)	25 (51.0%)	15 (57.7%)
Males	15 (48.4%)	22 (37.9%)	24 (49.0%)	11 (42.3%)
Mean Age at Onset, in months (standard deviation)	1.9 (3.3)	4.2 (4.0)	5.4 (7.9)	12.2 (12.5)
Mean Age at Last Evaluation, in years (standard deviation)	7.5 (5.2)	12.0 (7.7)	13.4 (10.1)	9.5 (5.9)

doi:10.1371/journal.pone.0127045.t001

Statistical Analysis

Descriptive analyses were used to examine the characteristics of the study population by mutation subtype. Since the age at onset was not normally distributed, quantile regression, more specifically median regression with gender adjustment, was used to estimate the differences in age at onset between E815K mutation, the D801N mutation and other mutations. Non-parametric survival analysis and log-rank tests were conducted to estimate and compare the curves of the cumulative probabilities of acquiring the gross motor skills of unsupported sitting and independent walking between the same mutation group pairs. Logistic regression was used to quantify how strongly the presence of an E815K mutation was associated with the occurrence of epilepsy. All the analyses were performed using the SAS version 9.2 statistical package (SAS Institute, Cary, NC). Statistical significance was assessed assuming a 0.05 significance level and a two-sided alternative hypothesis.

Results

Genetic analysis

We identified 34 distinct *ATP1A3* mutations in 154 patients, including 68 males and 86 females. One hundred forty three were simplex cases, 4 patients were two sets of monozygotic twins, and 7 patients were members of 2 families with dominant inheritance (Table 2). The mutations were all heterozygous and predominantly missense (31/34), located in the coding sequence of 9 exons of *ATP1A3*, with hot spots in exons 17 and 18 suggested by 3 recurrent common mutations. A deletion of 3 nucleotides in exon 20, predicting deletion of a single amino acid from the *ATP1A3* protein sequence, was found in 2 patients. Two intronic mutations at the donor site of exon 18 were found in 3 patients, predicting abnormal splicing of this intron (Fig 1). All the missense mutations identified in this cohort were predicted to be damaging (see S1 Table). We tested for their presence and frequency in the Kaviar database and concluded that these variants have never been observed other than in AHC patients. No mutation was detected in the exonic sequence of *ATP1A3* in 33 AHC patients.

The predominant mutation, D801N, was found in 40% of the simplex patients (58/143) and the mutation E815K in 26% (38/145); neither of these mutations were observed in multiplex cases (see S1 Table). In the 70 simplex cases for which DNA was available from both parents, all were confirmed to be de novo mutations. One dominant family with 4 affected family members was previously reported [3]. In another family, a heterozygous mutation (R756H) segregates with the disease phenotype in an affected mother and her two affected children. No *ATP1A3* mutation was found in 3 other multiplex families. Of the 34 mutations identified in this cohort, 16 are novel and 18 were previously reported in AHC patients [13–21].

Phenotype analysis and correlations

Data were extracted from standardized medical questionnaires that had been previously entered into a RedCAP database associated with the registry. Data for the current analysis were selected according to the reliability of documentation, their availability in most patients, and

Table 2. Summary of the 187 patients included in the genetic study.

Number of patients	Simplex cases	Homozygous twins	Multiplex cases	Total
With <i>ATP1A3</i> mutations	143	2 twins, 2 twins	4 of 1 family, 3 of 1 family	154
Without <i>ATP1A3</i> mutation	26	0	3 of 1 family, 2 of 1 family, 2 of 1 family	33
Total	169	4	14	187

doi:10.1371/journal.pone.0127045.t002

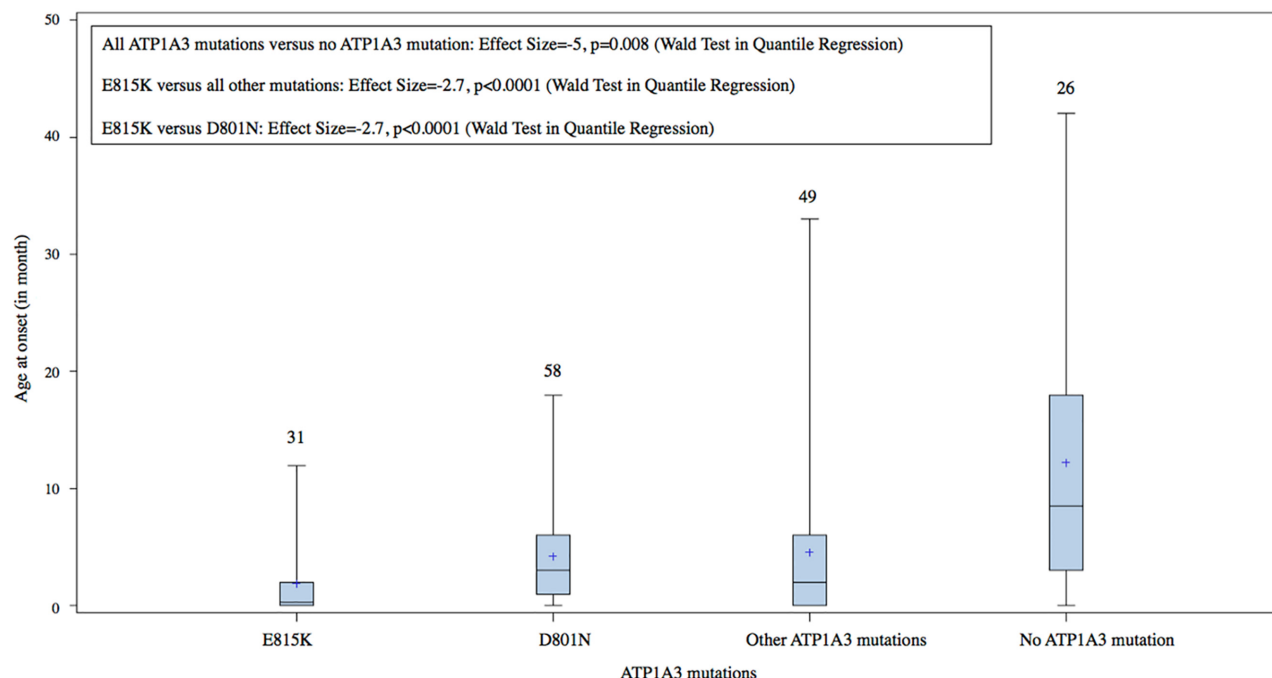


Fig 2. Ages at onset of AHC in each group of patients defined by their genotype. The horizontal lines in the boxes indicate the 25th percentile (bottom), the median (middle) and the 75 percentile (top) values. Crosses indicate the mean values. Numbers of patients analyzed in each group are indicated above the boxes.

doi:10.1371/journal.pone.0127045.g002

the protein. The 16 novel mutations we report here, however, effectively double the number of identified mutations associated with an AHC phenotype, and further extend the full range of identified mutations.

Although the majority of AHC mutations identified here did not overlap with mutations previously reported in association with an RDP phenotype, we found two mutations (D801Y

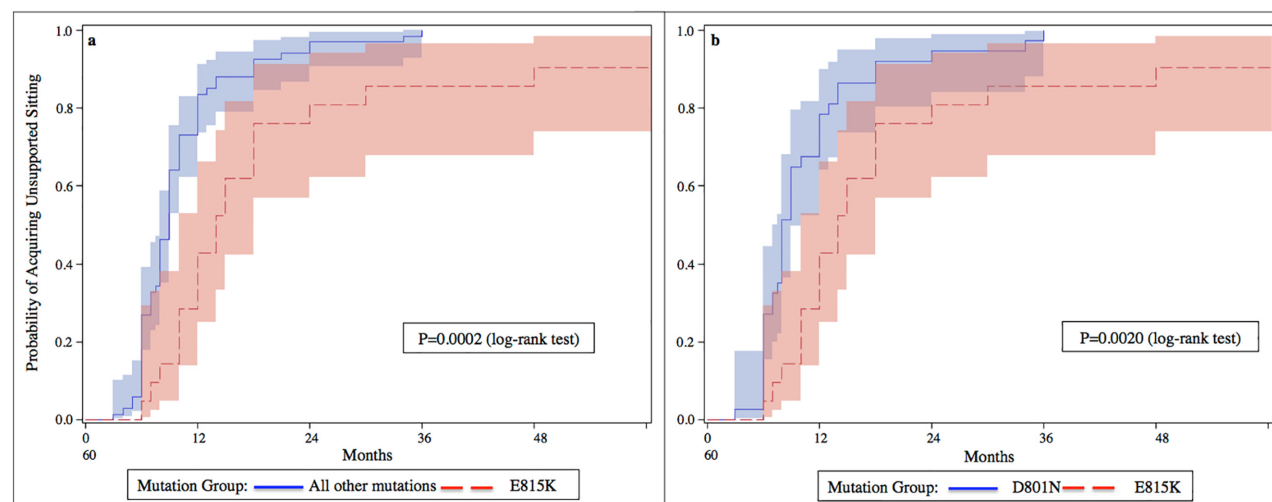


Fig 3. Ages at unsupported sitting acquisition in each group of patients defined by their genotype. Cumulative probability of acquiring unsupported sitting by patients presenting the E815K mutation, compared to patients mutation (3b). Patients with the E815K mutation are likely to gain unsupported sitting at a later age than patients in each of the other groups (respectively $P = 0.0002$ and $P = 0.0020$).

doi:10.1371/journal.pone.0127045.g003

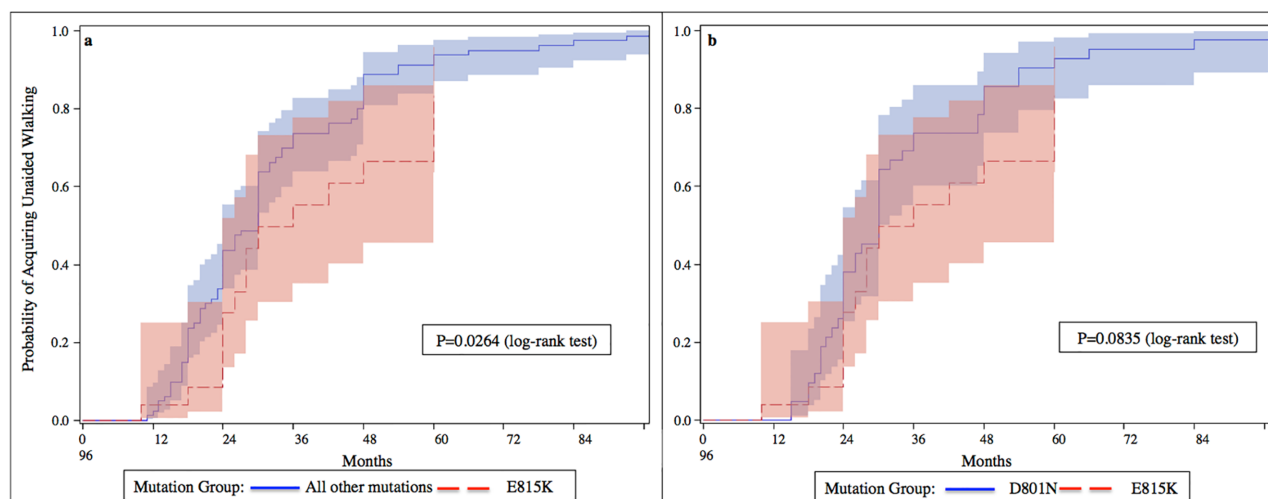


Fig 4. Ages at unaided walking acquisition in each group of patients defined by their genotype. Cumulative probability of acquiring unaided walking by patients presenting the E815K mutation, compared to patients with all other *ATP1A3* mutations (4a) and to patients with the D801N mutation (4b). Patients with the E815K mutation are likely to gain unaided walking at a later age than patients in each of the other groups (respectively $P = 0.0264$ and $P = 0.0835$).

doi:10.1371/journal.pone.0127045.g004

and R756H) previously reported with RDP phenotypes, although the latter familial occurrence has unique features, with some that overlap with both disorders. Clinical features of these patients are summarized in [S2 Table](#). Such variable expressivity has been recently reported for two other mutations (T370N and D923N) in *ATP1A3*-related disorders [19, 21]. Rosewich et al. noted that specific *ATP1A3* mutations were associated with certain features characteristic of both AHC and RDP phenotypes [19, 41].

Despite a high rate of mutation detection in *ATP1A3* in our study (82%), we did not identify mutations in all of the patients. Genome sequencing of the *ATP1A3* negative cohort also failed to identify obvious variants in genes associated with familial hemiplegic migraine (*ATP1A2*, *SLC1A3/EEAAT1*, *CACNA1A*), in agreement with prior investigations [18, 42–45].

Previous studies showed that patients with early onset of AHC symptoms tended to have a more severe clinical course [7, 11]. Our genotype-phenotype correlation study shows an earlier age at disease onset and a significantly higher incidence of status epilepticus in the E815K mutation group. Ishii et al. reported similar observations in Japanese patients with E815K mutations [14].

The significant delay in achievement of gross motor milestones observed here in patients with the most common *ATP1A3* mutations is also concordant with previous findings. However, patients within cohorts with the same mutation (for example, the 58 D801N-mutation patients and the 31 E815K-mutation patients with complete clinical data) presented a wide range of age of onset (0–12 months and 0–16 months, respectively), developmental delay, and overall neurologic morbidity. In comparing our E815K cohort to the Japanese E815K cohort,

Table 3. Odds ratio for occurrence of status epilepticus in AHC patients with different *ATP1A3* mutations.

ATP1A3 mutations	Odds Ratio	Confidence Interval	P-value
E815K vs. all other mutations	2.73	(1.166, 6.370)	0.021
E815K vs. D801N	1.12	(1.193, 7.919)	0.020
D801N vs. all other mutations	0.54	(0.252, 1.141)	0.106

doi:10.1371/journal.pone.0127045.t003

outcomes appear to be overall consistently more severe in the latter, suggesting the possible influence of epigenetic or additional unknown genetic factors that modify expression of the disease. However, we had less complete data for those with E815K than with D801N mutations in our cohort. Finally, we observed an earlier median age at onset in patients with any *ATP1A3* mutation (D801N, E815K or other) relative to patients without such mutations.

During the last 10 years, several functional studies have attempted to decipher the pathophysiology of *ATP1A3* related disorders and to explain the important phenotypical variability of these diseases [1, 46–53]. Using cellular models, Heinzen et al showed that D801N, E815K and 3 other AHC mutations did not decrease the level of expression of the alpha 3 subunit of the Na⁺/K⁺-ATPase but significantly decreased the ATPase activity [12]. This functional defect was quantitatively similar for E815K as for the other mutations, suggesting that the ATPase activity defect solely could not explain the higher severity of the E815K associated phenotype. The other major function of the alpha subunit of the Na⁺/K⁺ ATPase is the ion transport across the cell membrane, in a process called forward cycling: three Na⁺ go out of the cell while two K⁺ go in, using ATP hydrolysis, with a concomitant passive inward transport of protons [54]. Interestingly, the majority of the mutations associated with AHC are located in the transmembrane domains of the Na⁺/K⁺ ATPase alpha subunit, suggesting a specific impact of these mutations on ion binding and transport. Using electrophysiological techniques on *Xenopus* Levis oocytes, Li et al investigated the consequences of D801N, E815K and G947R mutations on Na⁺, K⁺ and H⁺ flows [55]. They showed that these 3 mutants were associated with a loss of ion transport with a strong dominant negative effect, suggesting that the loss of forward cycling is a pathological mechanism in AHC. The level of impact on Na⁺ and K⁺ transport was similar for D801N, E815K and G947R but the proton transport was significantly more profoundly reduced by the E815K than by the two other mutations. The severity of the phenotype associated with the E815K mutation is possibly the consequence of a more profound intracellular alkalosis. Intracellular proton concentration is known to be an important modulator of neuronal excitability and such a change may certainly have severe consequences on neuronal function.

Prediction of the course and severity of the disease using genotype information is a major aim of genotype-phenotype correlation studies. This information is essential for the evaluation of new therapeutics, to provide a more accurate prognosis to patients and caregivers, and to help develop the most appropriate guidelines for care management. Currently, however, the reliability and consistency of tools used to evaluate the severity of AHC are problematic. Panagiotakaki et al. designed specific indexes of paroxysmal and non-paroxysmal disability for AHC evaluation and used these tools in a 2-year retrospective and prospective study of 157 patients [10]. Still, this remarkable study did not demonstrate evidence of disease progression, in contrast to observations from the Japanese patient cohort [15]. Phenotype-genotype correlation from our analysis and others suggests that patients with E815K mutations are at greater risk of an acute clinical decompensation. Thus, even as our understanding of the genetics and pathophysiology of AHC increases, there is a continued need for comprehensive longitudinal natural history studies of AHC.

Conclusion

Further progress in our understanding of the pathophysiology of AHC and the broader spectrum of *ATP1A3* related phenotypes will require that a number of initiatives proceed in parallel, including 1) close collaboration among patients, physicians and researchers to facilitate early diagnosis via molecular studies; 2) early enrollment of the majority of patients in registries and prospective longitudinal studies; 3) a strong international collaboration to ensure uniform collection of data to better understand disease pathogenesis and outcome; and 4) the

identification and implementation of valid, reliable, and sensitive outcome measures for future use in clinical trials to assess promising therapies. Such outcomes measures should include (a) caregiver questionnaires and patient or caregiver reported outcomes, (b) standardized patient assessments of motor and cognitive function over a wide range of age and disease severity, and (c) tools for tracking AHC episode characteristics such as duration, frequency, and severity. Achieving such aims will enable progress towards an improved understanding of this complex and devastating disease, and help to ensure access to clinical trials and ultimately, truly effective therapies.

Supporting Information

S1 Table. Genetic study summary table. Heterozygous *ATP1A3* mutations and protein modifications found in AHC patients in the AHCF registry enrolled from 1997 to 2012. (PDF)

S2 Table. D801Y and R756H associated phenotypes. Summary of the clinical informations from the patients presenting the D801Y and the R756H mutations. (PDF)

Acknowledgments

We are grateful to the patients and families who have participated in these studies over nearly two decades. We are especially grateful to Sharon Ciccodicola and Lynn Egan of the US Alternating Hemiplegia of Childhood Foundation, Dominique Poncelin of the Association Française de l'Hémiplégie Alternante and Rosaria Vavassori of the Associazione Italiana per la Sindrome di Emiplegia Alternante for their leadership and support of this work. We gratefully acknowledge the many physicians who have referred patients to the registry over the past two decades, including Cigdem Akman (New York, NY), Dennis Altman (St. Louis, MO), Sheera Arakali (Pittsburgh, PA), Daniel Arndt (Aurora, CO), Todd Baran (Hershey, PA), Michael Baron (Milford, CT), Wendy Barton (Appleton, WI), David Bettis (Boise, ID), Peter Bingham (Burlington, VT), Blaise Bourgeois (Boston, MA), Marie Bourgeois (Paris, France), Galen Breningstall (Minneapolis, MN), Julia Brogli (Murray, Utah), David Burrows (Sault Ste Marie, Canada), Rebecca Campbell (Marshfield, WI), Robert Cerciello (West Hartford, CT), Denys Chaigne (Strasbourg, France), Bong Chang (Los Angeles, CA), Marie-Pierre Chaunu (Reims, France), Lan Chen (Los Angeles, CA), Claudia Chiriboga (New York City, NY), Ronald Christensen (Anchorage, AK), Robert Chudnow (Plano, TX), Keith Clayton (Provo, UT), Jay Cook (Galveston, TX), Dorothy Conselman (Kenosha, WI), Jeffery Corak (Deltona, FL), AJ Cottrell (Auckland County Durham, United Kingdom), Carl Crosley (Syracuse, NY), Steve Crouch (Topeka, KS), Thomas Dalrymple (Tallahassee, FL), Fernando Dangond (Boston, MA), Guillermo Davila-Gutierrez (Mexico D.F., Mexico), David Deloso (Mt. Vernon, IL), Orrin Devinsky (West orange, NJ), Marie-Emmanuelle Dilege (Montreal, QC), Francis Dimario (Hartford, CT), Nihal Olgac Dundar (Antalya, Turkey), Anne Dusser (Le Kremlin Bicêtre, France), Walter Ebecken (Niteroi, Brazil), Bernard Echenne (Montpellier, France), Frederick S Edelman (Madison, WI), Daniel Fain (Kalamazoo, MI), Kevin Farrell (Vancouver, Canada), Aviva Fattal-Valevski (Tel Aviv, Israel), Giorgio Felisari (Bosisio Parini, Italy), Raymond Fernandez (Tampa, FL), Leslie Fittenhoff (Alamo, CA), Blayne Fritz (Helena, MT), Antonio Gambardella (Catanzaro, Italy), Marion Gerard (Fontenay Sous Bois, France), Oded Gerber (East Setauket, NY), William Ginther (Akron, OH), Richard Gladstone (Toronto, Canada), Tracy Glauser (Cincinnati, OH), Ronald Goldman (Sonora, CA), Ronie Goldsmith (Concord, MA), Amy Goldstein (Pittsburgh, PA), Edward Goldstein (Atlanta GA), Francois Goutieres (Paris, France), Monty Grasmeder (Sitka, AK), Avram Greenspan (Redwood, CA), Matthew Grinsell

(Salt Lake City, Utah), Lilia Guerra (Lawrence, MA), Ajay Gupta (Cleveland, OH), Jin Hahn (Palo Alto, CA), Bradford Hale (Indianapolis, IN), Peter J Hand (Parkville, Australia), Joseph Hennessey (Huntingdon Valley, PA), Timothy Hoban (Ann Arbor, MI), John Huffstutter (Asheville NC), John Hutter (Tucson, AZ), Richard Jacobson (Milwaukee, WI), Ronald Jacobson (Sleepy Hollow, NY), Steven Janousek (Minneapolis, MN), Mary Johnston (Revelstoke, BC, Canada), Anna Kaminska (Paris, France), Allen Kaplan (Phoenix, AZ), Melvin Katz (North Brunswick, NJ), Paul Kazimiroff (West Los Angeles, CA), Nadir Khan (Racine, WI) David King (Halifax, Canada) Bruce Korf (Boston, MA), Andrew Kornberg (Melbourne, Australia), Rowena Korobkin (Oakland, CA), Cecile Laroche (Limoges, France), Richard Lavoie (Norwich, CT), Linda Leary (New York City, NY), Stephen Leiber (Ann Arbor, MI), Tali Lerman-Sagie (Holon, Israel), Mark Libenson (Boston, MA), Daniel Licht (Philadelphia, PA), Steven Lillpopp (Jacksonville, IL), Zhao Liu (Gainesville, FL), William Logan (Toronto, Canada), Alfredo Lohr Junior (Curitiba, Brazil), John Mantorani (St. Louis, MO), Cecile Marchal (Cedex, France), Henry Maguire (Danville, PA), Massimo Mastrangelo (Milan, Italy), Gerald Mayer (Yorktown Heights, NY), Margaret McBride (Rochester, NY), Tony McShane (Headington, United Kingdom), Kathleen McMahan (Metairie, LA), Brandon Meaney (Hamilton, Canada), Consuelo Mendoza (Brick, NJ), John H. Menkes (Beverly Hills, CA), Mohamad Mikati (Durham, NC), Steve Miller (Tulsa, OK), Leslie Morrison (Albuquerque, NM), Lakshmi Nacarajan (Perth Australia), Sanath Nallainathan (Stratford, CT), Juan Narbona (Pamplona, Spain), Mark Nespeca (San Diego, CA), Richard Nordgren (Lebanon, NH), Ann O'Hara (Aberdeen, Scotland), Donald Olson (Stanford, CA), Marcela Paredes (Santiago, Chile), Collette Parker (Jackson, MS), Jean-Marc Pedespan (Bordeaux, France), Marie-Jose Penniello (Caen, France), Cathy Pertureau (Montauban, France), Joseph Pinter (Davis, CA), J. George Quintana (Albuquerque, NM), Bolivar Fabien Quito Betancourt (Cuenca, Ecuador), Jill Ratner (Mount Kisco, NY), Christopher Reiner (Tampa, FL), George Reynolds (Houston, TX), Stephen D Rioux (Scarborough, ME), Carmen Roche (Madrid, Spain), Gabriel Ronen (Hamilton, Canada), Stanley Rothman (White Plains, NY), Barry Russman (Newington, CT), Joyce Sapin (Chalfont, PA), Benjamin Scheindlin (Burlington, MA), Gaston Schmidt (La Paz, Bolivia), Shlomo Shinnar (Bronx, NY), Naglaa Shourbaji (New Orleans, LA), Laufey Yr Sigurdardottir (Reykjavik, Iceland), Robert Simkins (Kettering, OH), Julius Simon (New Hartford, NY), Karl Smith (Durham, NC), Lindsay Smith (Clayton, Australia), Marjorie Smith (Anchorage, AK), Maria Sommerset (Poland, ME), Steven Sparagana (Dallas TX), Marc Starer (Burlington, VT), Iva Stepanova (Prague, Czech Republic), David Suhrbier (Pensacola, FL), Richard Sultan (Neptune, NJ), Weston Sumner (Albuquerque, NM), Kellie Sweat (Kalamazoo, MI), Carmela Tardo (New Orleans, LA), Jean E. Teasley (New Orleans, LA), Antonino Tedeschi (Reggio di Calabria, Italy), Elizabeth Thiele (Boston, MA), Joel Thompson, (Salt Lake City, Utah), Jerry Tomasovic (San Antonio, TX), Richard Torkelson (Kansas City, MO), Aley R Torres (Boston, MA), Vrajesh Udani (Mumbai, India), Paul Uvebrant (Sundbyberg, Sweden), Louis Vallee (Lille, France), Colin Van Orman (Salt Lake City, UT), Frederico Vigeveno (Rome, Italy), Stephen Weinstein (Washington D.C.), Paul R Weiner (Bethesda, MD), Arie Weinstock (Buffalo, NY), Karl Whener (San Angelo, TX), HT Wickremasinghe (Colombo, Sri Lanka), Angus Wilfong (Houston TX), Bonnie Williams (Dixon, IL), John K. Willis (New Orleans, LA), John Wilson (London, UK), Graham Wise (Randwick, Australia), Max Wiznitzer (Cleveland, OH), Steven Wolf (New York City, NY), John Wulff (Muncie, IN), Athina Xaidara (Athens, Greece), Sortiris Youroukos (Athens, Greece), and Arthur Zemach (Durango, CO).

Author Contributions

Conceived and designed the experiments: KJS LV. Performed the experiments: KJS LV GG KJM TMN SPR MS BN AJL MRM RT. Analyzed the data: LV GG DG LH BL JCR YZ CH LBJ.

Contributed reagents/materials/analysis tools: FA EA GA RLB CB MEB HTC SRC AC SDD JF MDK RJL MBM LJP RSR FR TDS MASDM PU MZ WX KS. Wrote the paper: LV KJS GG YZ KS.

References

1. Verret S, Steele JC. Alternating hemiplegia in childhood: a report of eight patients with complicated migraine beginning in infancy. *Pediatrics*. 1971; 47:675–680 PMID: [5089756](#)
2. Bourgeois M, Aicardi J, Goutieres F. Alternating hemiplegia of childhood. *J Pediatr*. 1993; 122:673–679 PMID: [8496742](#)
3. Mikati MA, Maguire H, Barlow CF, Ozelius L, Breakefield XO, Klauck SM, et al. A syndrome of autosomal dominant alternating hemiplegia: clinical presentation mimicking intractable epilepsy; chromosomal studies; and physiological investigations. *Neurology*. 1992; 42:2251–2257 PMID: [1361034](#)
4. 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; 45:833–6 PMID: [14667076](#)
5. Bassi MT, Bresolin N, Tonelli A, Nazos K, Crippa F, Baschiroto C, et al. A novel mutation in the ATP1A2 gene causes alternating hemiplegia of childhood. *J Med Genet*. 2004; 41:621–628 PMID: [15286158](#)
6. Swoboda KJ, Kanavakis E, Xaidara A, Johnson JE, Leppert MF, Schlesinger-Massart MB, et al. Alternating hemiplegia of childhood or familial hemiplegic migraine? A novel ATP1A2 mutation. *Ann Neurol*. 2004; 55:884–887 PMID: [15174025](#)
7. Mikati MA, Kramer U, Zupanc ML, Shanahan RJ. Alternating hemiplegia of childhood: clinical manifestations and long-term outcome. *Pediatr Neurol*. 2000; 23:134–141 PMID: [11020638](#)
8. Sakuragawa N. Alternating hemiplegia in childhood: 23 cases in Japan. *Brain Dev*. 1992; 14:283–288 PMID: [1456380](#)
9. Sweney MT, Silver K, Gerard-Blanluet M, Pedespan JM, Renault F, Arzimanoglou A, et al. Alternating hemiplegia of childhood: early characteristics and evolution of a neurodevelopmental syndrome. *Pediatrics*. 2009; 123:e534–541 doi: [10.1542/peds.2008-2027](#) PMID: [19254988](#)
10. Panagiotakaki E, Gobbi G, Neville B, Ebinger F, Campistol J, Nevsimalova S, et al. Evidence of a non-progressive course of alternating hemiplegia of childhood: study of a large cohort of children and adults. *Brain*. 2010; 133:3598–3610 doi: [10.1093/brain/awq295](#) PMID: [20974617](#)
11. 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*. 2012; 90:248–258
12. Heinzen EL, Swoboda KJ, Hitomi Y, Gurrieri F, Nicole S, de Vries B, et al. De novo mutations in ATP1A3 cause alternating hemiplegia of childhood. *Nat Genet*. 2012; 44:1030–1034 doi: [10.1038/ng.2358](#) PMID: [22842232](#)
13. Rosewich H, Thiele H, Ohlenbusch A, Maschke U, Altmüller J, Frommolt P, 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; 11:764–773 doi: [10.1016/S1474-4422\(12\)70182-5](#) PMID: [22850527](#)
14. Ishii A, Saito Y, Mitsui J, Ishiura H, Yoshimura J, Arai H, et al. Identification of ATP1A3 mutations by exome sequencing as the cause of alternating hemiplegia of childhood in Japanese patients. *PloS One*. 2013; doi: [10.1371/journal.pone.0056120](#)
15. Sasaki M, Ishii A, Saito Y, Morisada N, Iijima K, Takada S, et al. Genotype-phenotype correlations in alternating hemiplegia of childhood. *Neurology*. 2014; 82:482–490 doi: [10.1212/WNL.000000000000102](#) PMID: [24431296](#)
16. 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*. 2014; 18:50–54 doi: [10.1016/j.ejpn.2013.08.007](#) PMID: [24100174](#)
17. Ulate-Campos A, Fons C, Campistol J, Martorell L, Cancho-Candela R, Eiris J, et al. Alternating hemiplegia of childhood: ATP1A3 gene analysis in 16 patients. *Med Clin (Barc)*. 2014; 143:25–28
18. Yang X, Gao H, Zhang J, Xu X, Liu X, Wu X, et al. ATP1A3 Mutations and Genotype-Phenotype Correlation of Alternating Hemiplegia of Childhood in Chinese Patients. *PLoS One*. 2014; doi: [10.1371/journal.pone.0097274](#)
19. Rosewich H, Ohlenbusch A, Huppke P, Schlotawa L, Baethmann M, Carrilho I, et al. The expanding clinical and genetic spectrum of ATP1A3-related disorders. *Neurology*. 2014; 82:945–955 doi: [10.1212/WNL.0000000000000212](#) PMID: [24523486](#)

20. Vila-Pueyo M, Pons R, Raspall-Chaure M, Marcé-Grau A, Carreño O, Sintas C, et al. Clinical and genetic analysis in alternating hemiplegia of childhood: Ten new patients from Southern Europe. *J Neurol Sci.* 2014; 344:37–42 doi: [10.1016/j.jns.2014.06.014](https://doi.org/10.1016/j.jns.2014.06.014) PMID: [24996492](https://pubmed.ncbi.nlm.nih.gov/24996492/)
21. Heinzen EL, Arzimanoglou A, Brashear A, Clapcote SJ, Gurrieri F, Goldstein DB, et al. Distinct neurological disorders with ATP1A3 mutations. *Lancet Neurol.* 2014; 13:503–514. doi: [10.1016/S1474-4422\(14\)70011-0](https://doi.org/10.1016/S1474-4422(14)70011-0) PMID: [24739246](https://pubmed.ncbi.nlm.nih.gov/24739246/)
22. de Carvalho Aguiar P, Sweadner KJ, Penniston JT, Zaremba J, Liu L, Caton M, et al. Mutations in the Na⁺/K⁺-ATPase alpha3 gene ATP1A3 are associated with rapid-onset dystonia parkinsonism. *Neuron.* 2014; 43:169–175
23. Brashear A, Dobyns WB, de Carvalho Aguiar P, Borg M, Frijns CJ, Gollamudi S, et al. The phenotypic spectrum of rapid-onset dystonia-parkinsonism (RDP) and mutations in the ATP1A3 gene. *Brain.* 2007; 130:828–835 PMID: [17282997](https://pubmed.ncbi.nlm.nih.gov/17282997/)
24. Kamphuis DJ, Koelman H, Lees AJ, Tijssen MA. Sporadic rapid-onset dystonia-parkinsonism presenting as Parkinson's disease. *Mov Disord.* 2006; 21:118–119 PMID: [16161139](https://pubmed.ncbi.nlm.nih.gov/16161139/)
25. 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; 362:2213–2219 doi: [10.1056/NEJMcpc1002112](https://doi.org/10.1056/NEJMcpc1002112) PMID: [20558373](https://pubmed.ncbi.nlm.nih.gov/20558373/)
26. Kamm C, Fogel W, Wachter T, Schweitzer K, Berg D, Kruger R, et al. Novel ATP1A3 mutation in a sporadic RDP patient with minimal benefit from deep brain stimulation. *Neurology.* 2008; 70:1501–1503 doi: [10.1212/01.wnl.0000310431.41036.e0](https://doi.org/10.1212/01.wnl.0000310431.41036.e0) PMID: [18413579](https://pubmed.ncbi.nlm.nih.gov/18413579/)
27. 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; 22:1808–1809 PMID: [17595045](https://pubmed.ncbi.nlm.nih.gov/17595045/)
28. Linazasoro G, Indakoetxea B, Ruiz J, Van Blercom N, Lasa A. Possible sporadic rapid-onset dystonia-parkinsonism. *Mov Disord.* 2002; 17:608–609 PMID: [12112218](https://pubmed.ncbi.nlm.nih.gov/12112218/)
29. Pitttock SJ, Joyce C, O'Keane V, Hogle B, Hardiman MO, Brett F, et al. Rapid-onset dystonia-parkinsonism: a clinical and genetic analysis of a new kindred. *Neurology.* 2000; 55:991–995 PMID: [11061257](https://pubmed.ncbi.nlm.nih.gov/11061257/)
30. Svetel M, Ozelius LJ, Buckley A, Lohmann K, Brajković L, Klein C, et al. Rapid-onset dystonia-parkinsonism: case report. *J Neurol.* 2010; 257:472–474 doi: [10.1007/s00415-009-5385-y](https://doi.org/10.1007/s00415-009-5385-y) PMID: [19936820](https://pubmed.ncbi.nlm.nih.gov/19936820/)
31. Brashear A, Mink JW, Hill DF, Boggs N, McCall WV, Stacy MA, et al. ATP1A3 mutations in infants: a new rapid-onset dystonia-Parkinsonism phenotype characterized by motor delay and ataxia. *Dev Med Child Neurol.* 2012; 54:1065–1067 doi: [10.1111/j.1469-8749.2012.04421.x](https://doi.org/10.1111/j.1469-8749.2012.04421.x) PMID: [22924536](https://pubmed.ncbi.nlm.nih.gov/22924536/)
32. Zanolli-Fregonara P, Vidailhet M, Kas A, Ozelius LJ, Clot F, Hindie E, 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; 273:148–151 doi: [10.1016/j.jns.2008.06.033](https://doi.org/10.1016/j.jns.2008.06.033) PMID: [18675996](https://pubmed.ncbi.nlm.nih.gov/18675996/)
33. Blanco-Arias P, Einholm AP, Mamsa H, Concheiro C, Gutiérrez-de-Terán H, Romero J, 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; 18:2370–2377 doi: [10.1093/hmg/ddp170](https://doi.org/10.1093/hmg/ddp170) PMID: [19351654](https://pubmed.ncbi.nlm.nih.gov/19351654/)
34. Barbano RL, Hill DF, Snively BM, Light LS, Boggs N, McCall WV, et al. New triggers and non-motor findings in a family with rapid-onset dystonia-parkinsonism. *Parkinsonism Relat Disord.* 2012; 18:737–741 doi: [10.1016/j.parkreldis.2012.03.020](https://doi.org/10.1016/j.parkreldis.2012.03.020) PMID: [22534615](https://pubmed.ncbi.nlm.nih.gov/22534615/)
35. Kamm C, Fogel W, Wachter T, Schweitzer K, Berg D, Kruger R, et al. Novel ATP1A3 mutation in a sporadic RDP patient with minimal benefit from deep brain stimulation. *Neurology.* 2008; 70:1501–1503 doi: [10.1212/01.wnl.0000310431.41036.e0](https://doi.org/10.1212/01.wnl.0000310431.41036.e0) PMID: [18413579](https://pubmed.ncbi.nlm.nih.gov/18413579/)
36. Kamphuis DJ, Koelman H, Lees AJ, Tijssen MA. Sporadic rapid-onset dystonia-parkinsonism presenting as Parkinson's disease. *Mov Disord.* 2006; 21:118–119 PMID: [16161139](https://pubmed.ncbi.nlm.nih.gov/16161139/)
37. Ozelius LJ. Clinical spectrum of disease associated with ATP1A3 mutations. *Lancet Neurol.* 2012; 11:741–743 doi: [10.1016/S1474-4422\(12\)70185-0](https://doi.org/10.1016/S1474-4422(12)70185-0) PMID: [22857851](https://pubmed.ncbi.nlm.nih.gov/22857851/)
38. Rosewich H, Weise D, Ohlenbusch A, Gärtner J, Brockmann K. Phenotypic overlap of alternating hemiplegia of childhood and CAPOS syndrome. *Neurology.* 2014; 83:861–863. doi: [10.1212/WNL.0000000000000735](https://doi.org/10.1212/WNL.0000000000000735) PMID: [25056583](https://pubmed.ncbi.nlm.nih.gov/25056583/)
39. Demos MK, van Karnebeek CD, Ross CJ, Adam S, Shen Y, Zhan SH, et al. A novel recurrent mutation in ATP1A3 causes CAPOS syndrome. *Orphanet J Rare Dis.* 2014; doi: [10.1186/1750-1172-9-15](https://doi.org/10.1186/1750-1172-9-15)
40. Glusman G, Caballero J, Mauldin DE, Hood L, Roach JC. Kaviar: an accessible system for testing SNV novelty. *Bioinformatics.* 2011; 27:3216–3217 doi: [10.1093/bioinformatics/btr540](https://doi.org/10.1093/bioinformatics/btr540) PMID: [21965822](https://pubmed.ncbi.nlm.nih.gov/21965822/)
41. Rosewich H, Baethmann M, Ohlenbusch A, Gärtner J, Brockmann K. A novel ATP1A3 mutation with unique clinical presentation. *J Neurol Sci.* 2014; 341:133–135 doi: [10.1016/j.jns.2014.03.034](https://doi.org/10.1016/j.jns.2014.03.034) PMID: [24713507](https://pubmed.ncbi.nlm.nih.gov/24713507/)

42. De Vries B, Stam AH, Beker F, van den Maagdenberg AM, Vanmolkot KR, Lan L, et al. CACNA1A mutation linking hemiplegic migraine and alternating hemiplegia of childhood. *Cephalalgia*. 2008; 28:887–891 doi: [10.1111/j.1468-2982.2008.01596.x](https://doi.org/10.1111/j.1468-2982.2008.01596.x) PMID: [18498393](https://pubmed.ncbi.nlm.nih.gov/18498393/)
43. Jen JC, Wan J, Palos TP, Howard BD, Baloh RW. Mutation in the glutamate transporter EAAT1 causes episodic ataxia, hemiplegia, and seizures. *Neurology*. 2005; 65:529–534 PMID: [16116111](https://pubmed.ncbi.nlm.nih.gov/16116111/)
44. Vuillaumier-Barrot S, Panagiotakaki E, Le Bizec C, El Baba C, ENRAHs for SME Consortium, Fontaine B, et al. Absence of mutation in the SLC2A1 gene in a cohort of patients with alternating hemiplegia of childhood (AHC). *Neuropediatrics*. 2010; 41:267–269 doi: [10.1055/s-0031-1271767](https://doi.org/10.1055/s-0031-1271767) PMID: [21445818](https://pubmed.ncbi.nlm.nih.gov/21445818/)
45. De Vries B, Haan J, Stam AH, Vanmolkot KR, Stroink H, Laan LA, et al. Alternating hemiplegia of childhood: no mutations in the glutamate transporter EAAT1. *Neuropediatrics*. 2006; 37:302–304 PMID: [17236110](https://pubmed.ncbi.nlm.nih.gov/17236110/)
46. 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; 281:18539–18548 PMID: [16632466](https://pubmed.ncbi.nlm.nih.gov/16632466/)
47. Toustrup-Jensen MS, Holm R, Einholm AP, Schack VR, Morth JP, Nissen P, et al. The C terminus of Na⁺,K⁺-ATPase controls Na⁺ affinity on both sides of the membrane through Arg935. *J Biol Chem*. 2009; 284:18715–18725 doi: [10.1074/jbc.M109.015099](https://doi.org/10.1074/jbc.M109.015099) PMID: [19416970](https://pubmed.ncbi.nlm.nih.gov/19416970/)
48. Blanco-Arias P, Einholm AP, Mamsa H, Concheiro C, Gutiérrez-de-Terán H, Romero J, 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; 18:2370–2377 doi: [10.1093/hmg/ddp170](https://doi.org/10.1093/hmg/ddp170) PMID: [19351654](https://pubmed.ncbi.nlm.nih.gov/19351654/)
49. Clapcote SJ, Duffy S, Xie G, Kirshenbaum G, Bechard AR, Rodacker Shack V, 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; 106:14085–14090 doi: [10.1073/pnas.0904817106](https://doi.org/10.1073/pnas.0904817106) PMID: [19666602](https://pubmed.ncbi.nlm.nih.gov/19666602/)
50. 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; 285:26245–26254 doi: [10.1074/jbc.M110.123976](https://doi.org/10.1074/jbc.M110.123976) PMID: [20576601](https://pubmed.ncbi.nlm.nih.gov/20576601/)
51. Di Michele M, Goubau C, Waelkens E, Thys C, De Vos R, Overbergh L, 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 doi: [10.1016/j.jprot.2013.05.005](https://doi.org/10.1016/j.jprot.2013.05.005) PMID: [23681173](https://pubmed.ncbi.nlm.nih.gov/23681173/)
52. Weigand KM, Messchaert M, Swarts HG, Russel FG, Koenderink JB. Alternating Hemiplegia of Childhood mutations have a differential effect on Na⁺(+),K⁺(+)-ATPase activity and ouabain binding. *Biochim Biophys Acta*. 2014; 1842:1010–1016 doi: [10.1016/j.bbadis.2014.03.002](https://doi.org/10.1016/j.bbadis.2014.03.002) PMID: [24631656](https://pubmed.ncbi.nlm.nih.gov/24631656/)
53. Toustrup-Jensen MS, Einholm AP, Schack VR, Nielsen HN, Holm R, Sobrido MJ, et al. Relationship between intracellular Na⁺ concentration and reduced Na⁺ affinity in Na⁺,K⁺-ATPase mutants causing neurological disease. *J Biol Chem*. 2014; 289:3186–3197 doi: [10.1074/jbc.M113.543272](https://doi.org/10.1074/jbc.M113.543272) PMID: [24356962](https://pubmed.ncbi.nlm.nih.gov/24356962/)
54. Vedovato N, Gadsby DC. Route, mechanism, and implications of proton import during Na⁺/K⁺ exchange by native Na⁺/K⁺-ATPase pumps. *J Gen Physiol*. 2014; 143:449–464 doi: [10.1085/jgp.201311148](https://doi.org/10.1085/jgp.201311148) PMID: [24688018](https://pubmed.ncbi.nlm.nih.gov/24688018/)
55. Li M, Jazayeri D, Corry B, Melodi, McSweeney KM, Heinzen EL, Goldstein DB, et al. A functional correlate of severity in alternating hemiplegia of childhood. *Neurobiol Dis*. 2015; 77:88–93 doi: [10.1016/j.nbd.2015.02.002](https://doi.org/10.1016/j.nbd.2015.02.002) PMID: [25681536](https://pubmed.ncbi.nlm.nih.gov/25681536/)