variant, a relatively common variant in the Japanese population, was not observed in patients with multiple-system atrophy or controls in either the European or the North American series.

FUNCTIONAL ANALYSIS OF MUTANT COQ2

To determine the functional effect of each variant on the mitochondrial aerobic energy production in which coenzyme Q_{10} plays an essential

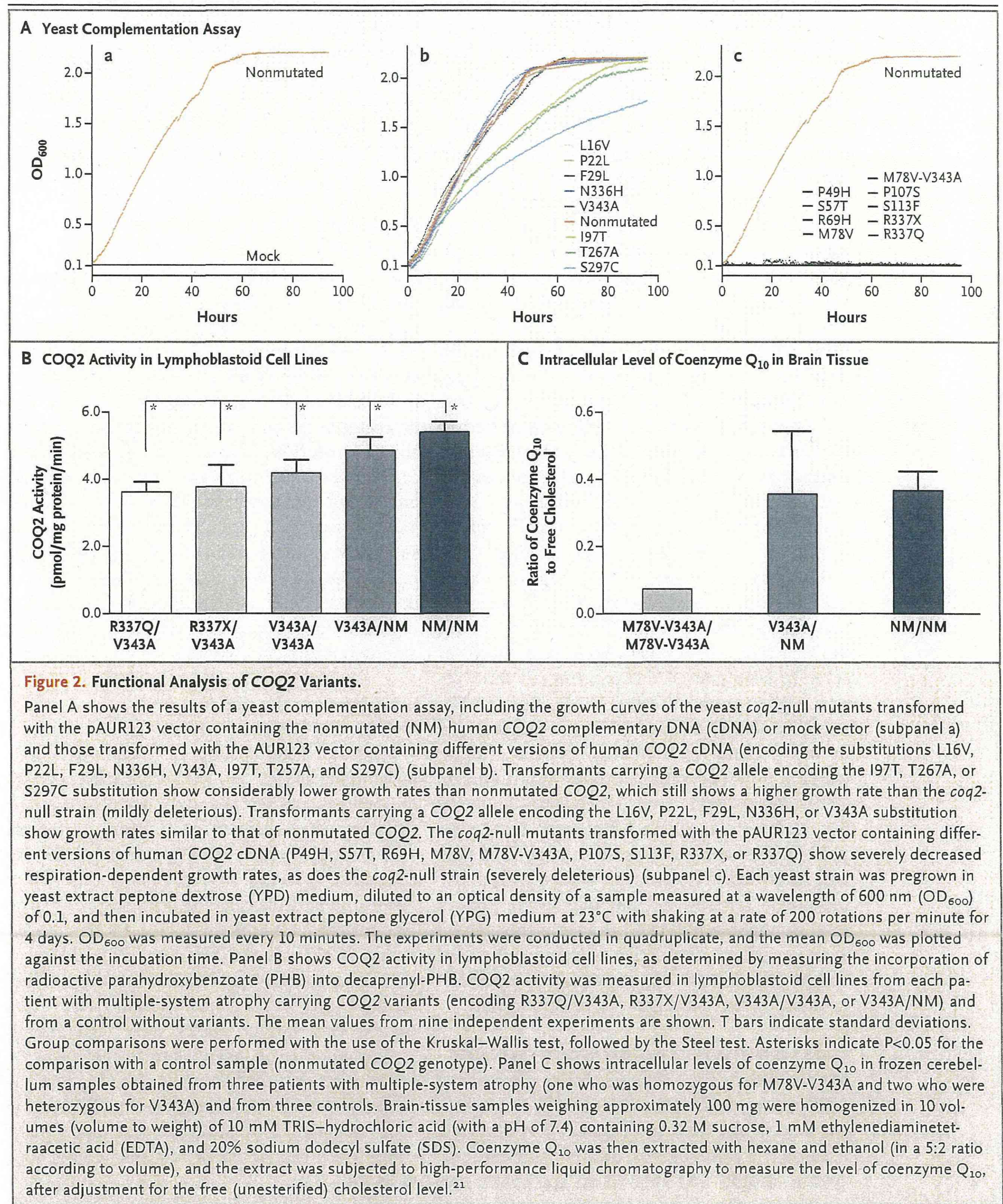

Figure 2. Functional Analysis of *COQ2* Variants.

Panel A shows the results of a yeast complementation assay, including the growth curves of the yeast *coq2*-null mutants transformed with the pAUR123 vector containing the nonmutated (NM) human *COQ2* complementary DNA (cDNA) or mock vector (subpanel a) and those transformed with the AUR123 vector containing different versions of human *COQ2* cDNA (encoding the substitutions L16V, P22L, F29L, N336H, V343A, I97T, T257A, and S297C) (subpanel b). Transformants carrying a *COQ2* allele encoding the I97T, T267A, or S297C substitution show considerably lower growth rates than nonmutated *COQ2*, which still shows a higher growth rate than the *coq2*-null strain (mildly deleterious). Transformants carrying a *COQ2* allele encoding the L16V, P22L, F29L, N336H, or V343A substitution show growth rates similar to that of nonmutated *COQ2*. The *coq2*-null mutants transformed with the pAUR123 vector containing different versions of human *COQ2* cDNA (P49H, S57T, R69H, M78V, M78V-V343A, P107S, S113F, R337X, or R337Q) show severely decreased respiration-dependent growth rates, as does the *coq2*-null strain (severely deleterious) (subpanel c). Each yeast strain was pregrown in yeast extract peptone dextrose (YPD) medium, diluted to an optical density of a sample measured at a wavelength of 600 nm (OD_{600}) of 0.1, and then incubated in yeast extract peptone glycerol (YPG) medium at 23°C with shaking at a rate of 200 rotations per minute for 4 days. OD_{600} was measured every 10 minutes. The experiments were conducted in quadruplicate, and the mean OD_{600} was plotted against the incubation time. Panel B shows COQ2 activity in lymphoblastoid cell lines, as determined by measuring the incorporation of radioactive parahydroxybenzoate (PHB) into decaprenyl-PHB. COQ2 activity was measured in lymphoblastoid cell lines from each patient with multiple-system atrophy carrying *COQ2* variants (encoding R337Q/V343A, R337X/V343A, V343A/V343A, or V343A/NM) and from a control without variants. The mean values from nine independent experiments are shown. T bars indicate standard deviations. Group comparisons were performed with the use of the Kruskal–Wallis test, followed by the Steel test. Asterisks indicate $P<0.05$ for the comparison with a control sample (nonmutated *COQ2* genotype). Panel C shows intracellular levels of coenzyme Q_{10} in frozen cerebellum samples obtained from three patients with multiple-system atrophy (one who was homozygous for M78V-V343A and two who were heterozygous for V343A) and from three controls. Brain-tissue samples weighing approximately 100 mg were homogenized in 10 volumes (volume to weight) of 10 mM TRIS–hydrochloric acid (with a pH of 7.4) containing 0.32 M sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA), and 20% sodium dodecyl sulfate (SDS). Coenzyme Q_{10} was then extracted with hexane and ethanol (in a 5:2 ratio according to volume), and the extract was subjected to high-performance liquid chromatography to measure the level of coenzyme Q_{10}, after adjustment for the free (unesterified) cholesterol level.[21]

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role in the electron transfer, we carried out functional complementation analysis by transforming the yeast *coq2*-null strain with nonmutated or mutated human *COQ2* cDNA (Fig. 2A). Transformants of the BY4741 Δ*coq2* yeast strain with the mutated *COQ2*, including transformants separately carrying the P49H, S57T, R69H, M78V, M78V-V343A, P107S, S113F, R337Q, and R337X alleles, showed severely decreased growth rates, similar to those observed in the *coq2*-null strain. In addition, transformants with mutated *COQ2*, including those with the variants encoding the I97T, T267A, and S297C substitutions, showed substantially lower growth rates than those expressing nonmutated *COQ2*, which had a higher growth rate than the *coq2*-null strain (mildly deleterious). The transformants with mutated *COQ2*, including transformants separately carrying the L16V, P22L, F29L, N336H, and V343A alleles, showed growth rates similar to those of the transformants expressing nonmutated *COQ2*. As described above, the yeast strain with M78V-V343A identified in Family 1 showed a severely decreased growth rate, whereas the strain with V343A had a growth rate similar to that of nonmutated *COQ2*, indicating that of the two variants, M78V primarily contributed to the impairment in COQ2 function.

Focusing on the rare variants that were identified in the case–control series (Table 1), we found that nine variants (P49H, S57T, R69H, I97T, P107S, S113F, T267A, S297C, and R337Q) were mildly or severely deleterious. On combining all three series, eight variants (P49H, S57T, I97T, P107S, S113F, T267A, S297C, and R337Q) were identified in 758 patients with multiple-system atrophy, whereas only one variant (R69H) was found in 1129 controls (odds ratio, 11.97; 95% CI, 1.60 to 531.52; P=0.004) (Table 2 footnote). Yeast complementation analysis showed that the F29L variant, identified in a European patient with multiple-system atrophy, did not impair the growth rate. Lymphoblastoid cell lines from this patient were unavailable for further measurement of the activity of mutant COQ2, thus making it difficult to interpret the pathogenicity of this variant.

COQ2 ACTIVITIES IN LYMPHOBLASTOID CELL LINES

We measured COQ2 activities in lymphoblastoid cell lines from patients carrying *COQ2* mutations, when available. We focused on the V343A variant because it is commonly associated with multiple-system atrophy and showed an apparently normal growth rate in the yeast complementation assay. We determined COQ2 activities in lymphoblastoid cell lines with *COQ2* variants R337Q/V343A, R337X/V343A, V343A/V343A, or V343A/NM and in a control without variants. The COQ2 activities in the lymphoblastoid cell lines (V343A/NM) obtained from patients with multiple-system atrophy were significantly lower than those in the control cell lines. The COQ2 activities in the cell lines from patients with multiple-system atrophy carrying two mutated *COQ2* alleles were further decreased (Fig. 2B).

CORRELATIONS BETWEEN GENOTYPE AND PHENOTYPE

The clinical features of patients with sporadic multiple-system atrophy carrying deleterious *COQ2* variants (as determined on yeast complementation assay and COQ2-activity measurement) and those of noncarriers are summarized in Table S5 in the Supplementary Appendix. The mean age at the onset of multiple-system atrophy among carriers was older than that among noncarriers (P=0.002). Among carriers, 34 had subtype C and 5 had subtype P. Among noncarriers, 468 had subtype C and 209 had subtype P. The subtype was unclassified in 42 noncarriers. The ratio of the number of patients with subtype C to the number with subtype P was significantly higher among carriers of *COQ2* variants than among noncarriers (P=0.02).

INTRACELLULAR COENZYME Q_{10} IN LYMPHOBLASTOID CELL LINES

We measured intracellular coenzyme Q_{10} levels in lymphoblastoid cell lines from patients with multiple-system atrophy and controls. The participants were grouped as follows: 3 patients with multiple-system atrophy carrying two variants (R337Q/V343A, R337X/V343A, and V343A/V343A), 16 patients carrying heterozygous V343A, 133 patients without variants, and 76 controls without *COQ2* variants (Table 3). Intracellular levels of coenzyme Q_{10} in lymphoblastoid cell lines from patients with multiple-system atrophy who carried two variant alleles were substantially lower than levels in cell lines from controls without variants. Intracellular coenzyme Q_{10} levels in patients who were heterozygous for V343A and in those without *COQ2* variants were not significantly lower than levels in controls without *COQ2* variants.

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Table 3. Intracellular Levels of Coenzyme Q_{10} in Lymphoblastoid Cell Lines, According to *COQ2* Variant.*

Variable	Patients with Multiple-System Atrophy					Controls
	R337Q/V343A	R337X/V343A	V343A/V343A	V343A/NM	NM/NM	NM/NM
No. of participants with variant	1	1	1	16	133	76
Ratio of coenzyme Q_{10} to free (unesterified) cholesterol†	2.19	2.58	1.86	3.38±0.53	3.41±0.74	3.48±0.75
Coenzyme Q_{10} level as a percentage of mean value in controls — %‡	62.9	74.1	53.4	97.1	98.0	100.0

* Plus–minus values are means ±SD. NM denotes nonmutated.
† The ratio of coenzyme Q_{10} to free (unesterified) cholesterol reflects the intracellular level of coenzyme Q_{10}. Lower values indicate decreased levels of intracellular coenzyme Q_{10}, presumably reflecting decreased biosynthesis of coenzyme Q_{10}. To calculate the ratio, coenzyme Q_{10} was measured in nanomoles per liter and free cholesterol in micromoles per liter.
‡ Lower values indicate decreased levels of intracellular coenzyme Q_{10}, as compared with the mean value in controls, presumably reflecting decreased biosynthesis of coenzyme Q_{10}.

COENZYME Q_{10} IN BRAIN TISSUE

Only a limited number of brain-tissue samples from patients with multiple-system atrophy carrying *COQ2* variants were available. Nevertheless, we measured coenzyme Q_{10} in frozen brain tissues from three patients with *COQ2* variants (one patient who was homozygous for M78V-V343A and two patients with V343A/NM) and from three controls without *COQ2* variants (Fig. 2C). The levels of coenzyme Q_{10} in patients who were homozygous for M78V-V343A were substantially lower than the levels in controls.

DISCUSSION

We identified homozygous or compound heterozygous *COQ2* mutations in two of the six multiplex families with multiple-system atrophy, a finding that suggests a role of these mutations in the pathogenesis of familial disease. We further found that functionally impaired variants in *COQ2* were associated with an increased risk of sporadic disease. In familial cases of multiple-system atrophy, linkage analysis strongly indicated locus heterogeneity in these families, and the identification of the causal variants in the remaining four families will require analyses such as whole-genome sequencing.

We found that a common variant (V343A) and multiple rare variants in *COQ2* were associated with sporadic multiple-system atrophy. The V343A variant was found exclusively in the Japanese participants, with an allele frequency of 1.6 to 2.2%. The allele frequency of V343A in patients with multiple-system atrophy (4.8%) was significantly higher than that in controls (1.6 to 2.2%) with odds ratios of 2.23 to 3.05. The modest risk of multiple-system atrophy that was associated with the common variant V343A suggests that V343A is a susceptibility factor rather than a causal factor for this disease. The odds ratio for the presence of deleterious rare variants was 11.97, which is much larger than that for V343A. Nonetheless, we should consider that these heterozygous variants in *COQ2* are not necessarily causal but rather confer a strong susceptibility to sporadic multiple-system atrophy. Members of Family 1 and Family 12 who carried deleterious variants in the heterozygous state did not have clinical signs of multiple-system atrophy.

The ratio of patients with subtype C multiple-system atrophy to those with subtype P was higher among carriers of deleterious *COQ2* variants than among noncarriers, which suggests that the cerebellum is more vulnerable to compromised COQ2 function than other regions of the central nervous system. Of the *COQ2* variants that we detected, the V343A variant was the most prevalent and was exclusively found in Japanese participants. These findings may in part explain the clinical observations that subtype C is more prevalent than subtype P in the Japanese population[9] but not in the European population[11] or the North American population.[12] However, there were only 35 carriers of deleterious *COQ2* variants among 363 patients with multiple-system atrophy in the Japanese case series. In addition, the clinical presentations of the two patients with familial

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disease who had the highest mutational load were different: subtype P in the patients in Family 1 and subtype C in the patients in Family 12. Thus, the genotypes of *COQ2* do not fully explain the clinical phenotypes.

Previous studies have shown evidence of mitochondrial respiratory-chain dysfunction or oxidative injury in patients with multiple-system atrophy.[22-24] The combination of oxidative stress and overexpression of oligodendroglial α-synuclein has been reported to replicate the characteristics of this disease.[25-28] Our findings suggest that impaired COQ2 activity, which would be predicted to impair the mitochondrial respiratory chain and increase vulnerability to oxidative stress, causes susceptibility to multiple-system atrophy. A primary deficiency of coenzyme Q_{10} that is caused by *COQ2* mutations has been described as an infantile-onset multisystem disorder and a nephropathy in several families.[29,30] The clinical presentation of these affected family members, however, differed markedly from the presentations of patients with multiple-system atrophy, perhaps because the decrease in COQ2 activity associated with the mutations in patients with multiple-system atrophy appears to be milder than that observed in patients with a primary deficiency of coenzyme Q_{10}.

Previous approaches to identifying susceptibility genes have used genomewide association studies or candidate-gene approaches.[31-33] Our identification of rare *COQ2* variants was accomplished by starting with multiplex families and then extending the analysis to patients with sporadic multiple-system atrophy, reflecting an alternative approach to the elucidation of genetic variants with strong effect sizes in an apparently nongenetic disorder.[34]

From the therapeutic viewpoint, oral supplementation with coenzyme Q_{10} may be helpful in treating multiple-system atrophy, particularly for patients with susceptibility-conferring *COQ2* variants. The safety and side-effect profile of high-dose supplementation with coenzyme Q_{10} have been well established.[35,36]

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Genotype–phenotype correlations in alternating hemiplegia of childhood

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Editorial, page 468

ABSTRACT

Objective: Clinical severity of alternating hemiplegia of childhood (AHC) is extremely variable. To investigate genotype-phenotype correlations in AHC, we analyzed the clinical information and *ATP1A3* mutations in patients with AHC.

Methods: Thirty-five Japanese patients who were clinically diagnosed with AHC participated in this study. *ATP1A3* mutations were analyzed using Sanger sequencing. Detailed clinical information was collected from family members of patients with AHC and clinicians responsible for their care.

Results: Gene analysis revealed 33 patients with de novo heterozygous missense mutations of *ATP1A3*: Glu815Lys in 12 cases (36%), Asp801Asn in 10 cases (30%), and other missense mutations in 11 cases. Clinical information was compared among the Glu815Lys, Asp801Asn, and other mutation groups. Statistical analysis revealed significant differences in the history of neonatal onset, gross motor level, status epilepticus, and respiratory paralysis in the Glu815Lys group compared with the other groups. In addition, 8 patients who did not receive flunarizine had severe motor deteriorations.

Conclusions: The Glu815Lys genotype appears to be associated with the most severe AHC phenotype. Although AHC is not generally seen as a progressive disorder, it should be considered a disorder that deteriorates abruptly or in a stepwise fashion, particularly in patients with the Glu815Lys mutation. *Neurology®* **2014;82:482–490**

GLOSSARY

AHC = alternating hemiplegia of childhood; **DYT12** = rapid-onset dystonia-parkinsonism.

Alternating hemiplegia of childhood (AHC) is a rare neurodevelopmental disorder characterized by recurrent flaccid or dystonic types of hemiplegic episodes lasting from several minutes to several days, abnormal ocular movements, involuntary movements, hypotonia, and seizures beginning in the infantile period (before 18 months of age).[1–4] Most patients have a sporadic form of the disorder, and routine laboratory and neuroimaging examinations do not show any specific abnormal findings.

ATP1A2 gene mutations have been reported as the cause of AHC in atypical familial cases.[5] However, these are rare. In 2012, 3 different research groups independently revealed that mutations of the sodium–potassium (Na^+/K^+)–ATPase $\alpha3$ subunit gene (*ATP1A3*) cause AHC.[6–8]

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ATP1A3 mutations have also been reported in rapid-onset dystonia–parkinsonism (DYT12).[9,10] Although the onset and clinical courses of these disorders are different, AHC and DYT12 may constitute a continuum of disorder;[6–8,11] therefore, there should be a variety of phenotypes of *ATP1A3*-related movement disorders.

Even in AHC alone, there are remarkable clinical variations among individuals.[12–16] Onset time, motor development levels, and cognition deficit levels differ considerably among individuals. Investigations among large populations in Europe and the United States have provided evidence of a nonprogressive course of AHC.[3,14,15] However, some degree of motor or intellectual deterioration has been observed in some patients with AHC.[13,16,17] Patients with early onset tend to have a severe clinical course.[16] We are unaware of the reason behind this clinical diversity in AHC. The position of the point mutations in *ATP1A3* and treatment methods used could be key factors.

METHODS Patients. ***Standard protocol approvals, registrations, and patient consents.*** Patients with AHC were recruited through the Japanese AHC Family Association. Thirty-four patients (8 female and 26 male) who were clinically diagnosed with AHC according to clinical diagnostic criteria[1–4] participated in the study with ages ranging from 1 year 4 months to 43 years. Another male patient who did not fulfill the criteria (onset at 2 years of age) was also enrolled in the study. All patients had sporadic AHC. Most of the parents participated in the study.

Ethics statement. This study was approved by the Ethics Review Committee of Fukuoka University. The parents of the patients provided informed consent before the start of the study.

Table 1 ***ATP1A3* mutations and protein modifications in patients with alternating hemiplegia of childhood**

Exon	Nucleotide change	Amino acid change	Number (%) of probands
5	410 C>A	Ser137Tyr (S137Y)	1
9	1072 G>T	Gly358Cys (G358C)	1
16	2263 G>T	Gly755Cys (G755C)	1
16	2263 G>A	Gly755Ser (G755S)	1
16	2264 G>C	Gly755Ala (G755A)	1
17	2312 C>A	Thr771Asn (T771N)	1
17	2401 G>A	Asp801Asn (D801N)	10 (30)
18	2443 G>A	Glu815Lys (E815K)	12 (36)
20	2767 G>A	Asp923Asn (D923N)	1
20	2780 G> T	Cys927Phe (C927F)	2
21	2839 G>C	Gly947Arg (G947R)	1
22	2974 G>T	Asp992Tyr (D992Y)	1
Total			33

We collected detailed clinical information regarding the onset time of the initial symptoms, frequency and type (flaccid or dystonic) of recurrent hemiplegic attacks, frequency of seizures, experience of status epilepticus and respiratory paralysis, involuntary movements, developmental history, level of gross motor development, and cognitive function in the intermittent period between recurrent hemiplegic attacks and flunarizine usage (particularly age at initiation, dose, continuation, and age at which drug was stopped if appropriate) from the parents of patients with AHC and clinicians (primarily pediatric neurologists) responsible for their care through an intake form. All participants except one boy (onset at 2 years of age) were confirmed to fulfill the AHC criteria and were subsequently screened for *ATP1A3* mutations.

Mutation analysis. Sanger sequencing was performed to analyze genomes of the patients and their parents. Genomic DNA was prepared from EDTA-Na_2-containing blood samples using a QIAamp DNA Blood Maxi Kit (Qiagen, Hilden, Germany) according to the protocol provided by the manufacturer. All of the exons and intron–exon boundaries of *ATP1A3* were amplified by PCR using the designed PCR primers. The primer sequences and PCR conditions are available upon request. PCR products were purified in ExoSAP-IT for PCR Product Clean-Up (Affymetrix, Santa Clara, CA) with 1 cycle of 15 minutes at 37°C and another of 15 minutes at 80°C. The purified PCR products were sequenced using the ABI PRISM BigDye 3.1 terminator method (Applied Biosystems, Foster City, CA) and an ABI PRISM® 3100 Genetic Analyzer (Applied Biosystems). We also recruited 96 unrelated healthy Japanese volunteers who were free of seizures or without any history of epilepsy as a control group.

Before the present study, we attempted to identify the genes responsible for AHC by whole-exome sequencing analysis (using a new generation sequencer) of 8 Japanese patients with AHC who were clinically diagnosed with typical AHC. This previous study revealed heterozygous missense mutations in *ATP1A3* in all of the 8 patients studied.[8] Subsequently, we continued our *ATP1A3* analyses using Sanger sequencing of 35 Japanese patients with AHC (including the 8 patients).

Evaluation of clinical information and statistical analysis. We compared the relationship between the point mutations in *ATP1A3* and clinical information. All the data analyses were performed using the SAS software package (version 9.2; SAS Institute Inc., Cary, NC). Frequency distributions of the phenotypes were evaluated using Fisher exact test.

RESULTS Gene mutations. A heterozygous missense mutation in *ATP1A3* was confirmed in 33 of the 35 patients by Sanger sequencing. Thirty-three (7 female and 26 male) of the 35 patients were observed to have a heterozygous missense mutation. The rate of genetic mutation was as high as 94%. None of the parents showed any *ATP1A3* mutations. All mutations were thus confirmed as de novo mutations. Of the 33 patients with *ATP1A3* mutations, 12 (36%) had a c.2443 G>A, p.Glu815Lys (E815K) mutation; 10 patients (30%) had a c.2401 G>A, p.Asp801Asn (D801N) mutation; 2 patients (7%) had a c.2780 G>T, Cys927Phe (C927F) mutation; and the remaining 9 patients had other mutations. There were 3 Gly755 mutations: c.2263 G>T, p.Gly755Cys (G755C); c.2263 G>A, p.Gly755Ser (G755S); and c.2264 G>C, p.Gly755Ala (G755A) (table 1).

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The patient who experienced disease onset at age 2 had an Asp923Asn (D923N) mutation.

Clinical features. We divided the patients into 3 groups based on the *ATP1A3* mutations. Group 1 included patients with an E815K mutation (12 cases), group 2 those with a D801N mutation (10 cases), and group 3 those with other mutations (11 cases). The clinical information from all of the patients (table 2 and table 3) was compared among these groups. Distinct differences in several of the items were observed in group 1 compared with the other groups (table 4).

In group 1 (E815K mutation), the onset time of abnormal ocular movements or seizures was during

Table 2 First symptoms of onset and development in each patient

Case number	Age, y	Sex	*ATP1A3* mutation	Onset times and symptoms	First hemiplegic attack	Head control	Sitting	Stand with support	Unassisted walk	Run
G-1-01	33	F	E815K	2 d; abnormal eye movements	1 y, 0 mo	2 y, 0 mo	2 y, 6 mo	3 y, 0 mo	Impossible	
G-1-02	16	M	E815K	0 d; convulsion	1 y, 3 mo	1 y, 0 mo	1 y, 9 mo	Impossible		
G-1-03	14	M	E815K	1 mo; abnormal eye movements, convulsion	5 mo	7 mo	1 y, 0 mo	1 y, 6 mo	Impossible	
G-1-04	14	M	E815K	17 d: abnormal eye movements, convulsion	10 mo	7 mo	9 mo	2 y, 2 mo	Impossible	
G-1-05	14	M	E815K	1 d; abnormal eye movements	5 mo	1 y, 6 mo	1 y, 10 mo	2 y, 6 mo	Impossible	
G-1-06	12	M	E815K	1 d; abnormal eye movements	3 mo	8 mo	1 y, 0 mo	Impossible		
G-1-07	9	M	E815K	1 d; abnormal eye movements, convulsion	10 mo	2 y	3 y, 6 mo	4 y, 6 mo	Impossible	
G-1-08	9	M	E815K	2 d; abnormal eye movements	8 mo	2 y, 6 mo	3 y, 3 mo	8 y	Impossible	
G-1-09	7	M	E815K	0 d; abnormal eye movements, convulsion	4 mo	Impossible				
G-1-10	4	M	E815K	0 d; abnormal eye movements	6 mo	1 y, 0 mo	1 y, 6 mo	1 y, 10 mo	Impossible	
G-1-11	1 y, 6 mo	M	E815K	1 d; convulsion	7 mo	Impossible				
G-1-12	1 y, 4 mo	M	E815K	0 d; abnormal eye movements	9 mo	Impossible				
G-2-01	43	F	D801N	10 mo; dystonic hemiplegia	10 mo	5 mo	1 y, 0 mo	No record	3 y	5 y
G-2-02	33	F	D801N	3 mo; flaccid paralysis	3 mo	4 mo	7 mo	No record	5 y, 5 mo	Impossible
G-2-03	25	M	D801N	1 mo; abnormal eye movements	9 mo	4 mo	7 mo	11 mo	5 y	Impossible
G-2-04	20	M	D801N	1 d; convulsion	4 mo	5 mo	9 mo	1 y, 0 mo	2 y, 3 mo	3 y, 6 mo
G-2-05	19	M	D801N	2 mo; convulsion	2 mo	5 mo	10 mo	12 mo	Impossible	
G-2-06	18	F	D801N	4 mo; abnormal eye movements	4 mo	6 mo	No record	2 y	3 y, 6 mo	Impossible
G-2-07	13	M	D801N	0 d; abnormal eye movements	9 mo	6 mo	11 mo	3 y, 10 mo	Impossible	
G-2-08	12	M	D801N	4 mo; hemidystonia	9 mo	5 mo	7 mo	1 y, 10 mo	4 y, 6 mo	6 y
G-2-09	12	M	D801N	6 mo; hemidystonia	6 mo	3 mo	9 mo	10 mo	3 y, 3 mo	Impossible
G-2-10	3	M	D801N	5 mo; hemidystonia	5 mo	3 mo	1 y, 2 mo	1 y, 3 mo	Impossible	
G-3-01	30	F	S137Y	1 mo; seizure	6 mo	5 mo	2 y, 0 mo	4 y, 1 mo	Impossible	
G-3-02	25	M	G755A	2 mo; abnormal ocular movements	6 mo	1 y, 0 mo	2 y, 0 mo	3 y, 0 mo	Impossible	
G-3-03	24	M	T771N	5 mo; abnormal ocular movements, seizure	1 y, 0 mo	5 mo	9 mo	11 mo	2 y, 0 mo	3 y
G-3-04	23	M	D992Y	8 mo; abnormal ocular movements	8 mo	No record	No record	1 y, 5 mo	2 y, 10 mo	4 y
G-3-05	18	M	G755C	2 mo; abnormal ocular movements	10 mo	6 mo	1 y	1 y, 6 mo	3 y	5 y
G-3-06	17	F	C927F	2 mo; abnormal ocular movements	1 y, 0 mo	4 mo	7 mo	10 mo	2 y, 2 mo	4 y
G-3-07	13	M	G755S	3 mo; abnormal ocular movements	4 mo	4 mo	7 mo	1 y, 2 mo	Impossible	
G-3-08	12	F	C927F	2 mo; abnormal ocular movements	3 y	3 mo	5 mo	No record	1 y, 10 mo	3 y
G-3-09	11	M	D923N	2 y; left hemiplegia	2 y	3 mo	6 mo	No record	1 y, 2 mo	1 y, 8 mo
G-3-10	8	M	G358C	1 d; seizure	1 mo	7 mo	Impossible			
G-3-11	2 y, 8 mo	M	G947R	2 d; seizure	8 mo	4 mo	No record	8 mo	2 y, 8 mo	Impossible

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