Genotype–phenotype correlations in alternating hemiplegia of childhood

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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 α3 subunit gene (*ATP1A3*) cause AHC.[6–8]

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Go to Neurology.org for full disclosures. Funding information and disclosures deemed relevant by the authors, if any, are provided at the end of the article.

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.

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.

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

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).

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

Table 3 Details of clinical symptoms in each patient

Case number	Flunarizine	Motor deterioration; trigger	Cognitive deficit	Convulsion (status epilepticus)	Frequency of hemiplegia/mo	Respiratory paralysis	Respirator care	Tube feeding
G-1-01	Not used	+ At 6 y: stand→bedridden; fever	Profound	+	Flaccid, continuous	+	RC, T	TC
G-1-02	Discontinued at 5 y	+ At 6 y: stand→bedridden; fever	Sentence	+	Flaccid 25	+		
G-1-03	15 mg	No	Words	+	Flaccid 10	+		
G-1-04	Discontinued at 6 y	+ At 7 y: stand→bedridden; status epilepticus	Words	+	Flaccid 15, dystonic 1	+	RB	TB
G-1-05	Discontinued at 3 y	+ At 4 y: stand→bedridden	Profound	+	Flaccid, continuous	+	RB, T	TC
G-1-06	Not used	+ At 3 y: sit→bedridden; status epilepticus	Profound	+	Flaccid 2	+	RB	TC
G-1-07	5 mg	No	Words	+	Flaccid 10, dystonic 30	+		
G-1-08	12.5 mg	+ At 8 y: stand→sit; unknown	Sentence	+	Flaccid 10	+		
G-1-09	5 mg	No	Severe	+	Flaccid 30	+	RB	
G-1-10	Not used	No	Words	+	Flaccid 4	+		
G-1-11	Not used	No	Severe	+	Flaccid 30	+		
G-1-12	7.5 mg	No	Severe	+	Flaccid 15	+		TC
G-2-01	Discontinued at 16 y	No at 16 y: long-lasting left hemidystonia	Sentence	−	Flaccid 1, dystonic 2	−		
G-2-02	5 mg	No	Sentence	−	Flaccid 6, dystonic 2	−		
G-2-03	10 mg	No	Sentence	−	Flaccid 12, dystonic 8	−		
G-2-04	Discontinued at 9 y	No	Sentence	−	Flaccid 10	−		
G-2-05	Discontinued at 5 y	No	Sentence	+	Flaccid 3	−		
G-2-06	30 mg	No	Sentence	−	Flaccid 15	−		
G-2-07	5 mg	No	Words	+	Flaccid, dystonic 4	+		
G-2-08	10 mg	No at 3 y: long-lasting left hemidystonia	Sentence	−	Flaccid 8, dystonic continuous	+		
G-2-09	Discontinued at 3 y	No	Sentence	−	Flaccid 25	−		
G-2-10	5 mg	No at 11 mo: long-lasting left hemidystonia	Words	−	Flaccid 2, dystonic 4	+		TB
G-3-01	Discontinued at 16 y	+ At 17 y: stand→bedridden; status epilepticus	Profound	+	Flaccid, continuous	+	RB	TC
G-3-02	Discontinued at 15 y	+ At 16 y: stand→bedridden; status epilepticus	Words	+	Flaccid 1	+		
G-3-03	Discontinued at 12 y	+ At 13 y: run→walk; unknown	Sentence	−	Flaccid 5, dystonic 2	−		
G-3-04	5 mg	No	Sentence	−	Flaccid 2, dystonic 10	−		
G-3-05	5 mg	No	Sentence	+	Flaccid 2	+		
G-3-06	Discontinued at 3 y	No	Sentence	−	Flaccid 15	−		
G-3-07	Discontinued at 5 y	+ At 12 y: stand→bedridden; status epilepticus	Profound	+	Flaccid, continuous	+	RC, T	TC
G-3-08	5 mg	No	Sentence	−	Flaccid 1, dystonic 2	−		
G-3-09	5 mg	No	Sentence	−	Dystonic 10	−		
G-3-10	5 mg	No	Profound	+	Flaccid 5	+	RB	
G-3-11	5 mg	No	Words	−	Flaccid 2, dystonic 2	−		

Abbreviations: B = before; C = continue; R = respirator; T = tracheostomy.

Table 4 Comparison between group 1 vs group 2, group 3, and groups 2 + 3

	Group 1 (n = 12), n (%)	Group 2 (n = 10), n (%)	*p* Value (group 1 vs 2)	Group 3 (n = 11), n (%)	*p* Value (group 1 vs 3)	Groups 2 + 3 (n = 21), n (%)	*p* Value (group 1 vs 2 + 3)
Neonatal onset	11 (91.7)	2 (20)	0.0015[a]	2 (18.2)	0.0006[a]	4 (19.0)	0.0001[a]
Unassisted walking	0 (0.0)	7 (70)	0.0007[a]	7 (63.6)	0.0013[a]	14 (66.7)	0.0002[a]
Prolonged severe motor deterioration	5 (41.7)	0 (0)	0.040[b]	3 (27.3)	0.67	3 (14.2)	0.11
Cognitive deficit, severe or profound	8 (66.7)	0 (0)	0.0017[a]	3 (27.3)	0.10	3 (14.2)	0.0055[a]
Status epilepticus	12 (100)	2 (20)	0.0001[a]	5 (45.5)	0.0046[a]	7 (33.3)	0.0002[a]
Respiratory paralysis	12 (100)	3 (30)	0.0007[a]	5 (45.5)	0.0046[a]	8 (38.0)	0.0005[a]
Respirator care	5 (41.7)	0 (0)	0.040[b]	3 (27.3)	0.67	3 (14.2)	0.11
Tube feeding	5 (41.7)	1 (10)	0.16	2 (18.2)	0.37	3 (14.2)	0.11

Group 1: E815K mutation; group 2: D801N mutation; group 3: other mutations.
[a] Statistically significant ($p < 0.01$) (Fisher exact test).
[b] Statistically significant ($p < 0.05$) (Fisher exact test).

the neonatal period (less than 7 days after birth) in 11 of the 12 patients. The first symptom was observed in the last patient at 1 month of age. All patients showed very slow early development. None of the patients was able to control head movements before 6 months of age. Three patients did not develop head control at all, although they could all roll over during the interictal period.

The peak motor development was identified as "standing with support" in 7 patients. None of the patients in group 1 could walk independently, even in the interictal period between recurrent hemiplegic attacks (figure, A). All 12 patients experienced both status epilepticus and respiratory paralysis, and most had visited emergency rooms of hospitals. Five patients experienced a permanent severe motor deterioration from sitting or standing with support to becoming bedridden in childhood. All 5 patients experienced this severe deterioration: the condition of 3 patients deteriorated because of status epilepticus and that of the remaining 2 patients deteriorated because of recurrent fever. Four of these patients were immediately treated by the emergency hospitals using mechanical respiratory care. Thereafter, 2 patients were placed under all-day respiratory care. In these 5 patients, only mild brain atrophy or mild cerebellar atrophy was revealed by brain MRI. Tube feeding was required in 4 patients. Out of the 5 patients in whom severe deterioration was observed, 4 had discontinued flunarizine before the severe deterioration occurred and the remaining patient was not administered flunarizine.

In group 2 (D801N mutation), the onset time was during the neonatal period in only 2 of the 10 patients. Patients in this group were characterized by slower than normal early development, but all patients were able to control their head movements by 6 months of age. Seven patients could walk independently in the interictal period between hemiplegic attacks (figure, B). Three patients experienced several episodes of hemidystonia lasting for several weeks to a few months. None of the patients showed severe motor deterioration. All 10 patients were treated with flunarizine. Four patients had discontinued flunarizine more than 10 years previously, but they showed no severe motor deterioration.

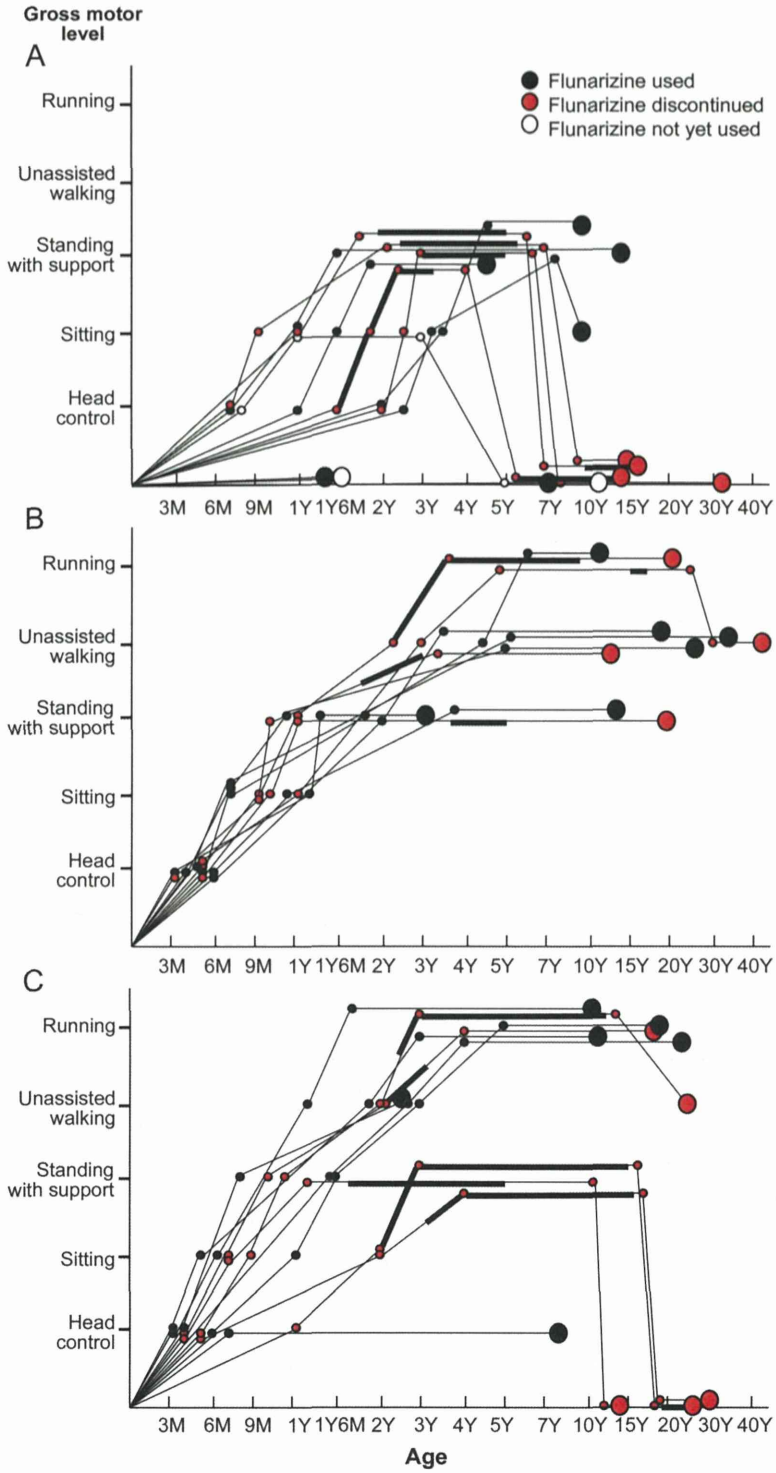
In group 3 (other mutations), the onset time was during the neonatal period in only 2 of the 11 patients. Most patients in this group showed slight delays in early development, and 7 of the 11 patients were able to walk unassisted in the interictal period between recurrent hemiplegic attacks. Six of these 7 patients had no obvious signs of motor deterioration. However, 3 patients who could stand with support abruptly experienced a severe motor deterioration in their teens (figure, C). All 11 patients were treated with flunarizine, and 3 of the 5 patients who discontinued flunarizine treatment showed permanent severe deterioration after status epilepticus; these 3 patients had the following *ATP1A3* mutations: S137Y, G755A, and G755S.

Severe motor deterioration after status epilepticus or fever during childhood was observed in 5 of the 12 patients with an E815K mutation and 3 of the 11 patients with other mutations.

Regarding flunarizine usage, 31 of the 33 patients with *ATP1A3* mutations were administered flunarizine, and this was discontinued in 13 patients. Seven of the 13 patients who discontinued flunarizine experienced either an abrupt or stepwise severe motor deterioration. In addition, 8 patients with severe motor deterioration had not been administered flunarizine during the period of deterioration. No patient who continued flunarizine showed severe motor deterioration.

The patient with the D923N mutation showed normal motor development.

Figure History of gross motor development during the intermittent period for each patient



(A) E815K mutation group (group 1), (B) D801N mutation group (group 2), (C) other mutations group (group 3). Small circles: Age at which gross motor development was attained or lost. Large circles: Age of each patient during the study. Thin lines: Gross motor level ignoring short-term fluctuations. Thick lines: Period of flunarizine administration among patients for whom flunarizine treatment was discontinued.

Statistical analysis. Statistical analyses revealed significant differences between group 1 and the other groups in terms of neonatal onset, unassisted walking, severe cognitive deficit, and history of status epilepticus and respiratory paralysis (table 4). Group 1 was shown to have a more severe phenotype than the other groups.

DISCUSSION Similar to patients in Europe and the United States, *ATP1A3* genetic analysis revealed that E815K (36%) and D801N (30%) mutations are common in Japanese patients with AHC. Reasons for male/female ratio deviation in this study were unclear. Because a male bias is not typical of AHC, it is possible that some female patients may have not yet been diagnosed in Japan.

We observed that the E815K mutation group had more severe symptoms than the other mutation groups with respect to 1) onset time of the first symptoms, 2) unassisted walking, 3) cognitive deficit, 4) status epilepticus, and 5) respiratory paralysis. Although the number of participants was relatively small, this study demonstrated that the E815K mutation was associated with the most severe AHC phenotype. The D801N mutation possibly results in a moderate to mild form of AHC. Some other mutations, such as G755A, G755S, and S137Y, may also result in a severe phenotype, but the rest of "other mutations" identified in this study could result in a relatively mild phenotype. The reason why the early-onset group tended to show a more severe clinical course[16] could be partly explained by the findings of this study.

Previous studies have not been able to establish any genotype–phenotype correlations in patients with AHC.[6,7] However, one study of 24 patients reported a tendency for AHC patients with an E815K mutation to have a more severe subtype than those with a D801N mutation because 1) only 2 of the 7 patients with an E815K mutation had a peak motor function of "walking unassisted" compared with 8 of the 9 patients with a D801N mutation, 2) progression of nonparoxysmal features was seen in 4 of the 7 patients with an E815K mutation but only in 1 of the 9 patients with a D801N mutation, and 3) muscular hypotonia was seen in all 7 patients with an E815K mutation but only in 5 of the 9 patients with a D801N mutation.[7] These findings support our observation that the E815K mutation results in a more severe AHC phenotype. However, a larger study of 82 patients demonstrated no genotype–phenotype correlations in AHC.[6] Our positive findings may thus be because of our relatively small sample size.

Severe deterioration or sudden death have long been associated with AHC.[14,16,17] Permanent loss of function has sometimes been reported after a severe episode, which is a major concern for many families.[17] However, it has been suggested that AHC is probably not an intrinsically progressive disease, but one that can show stepwise deterioration with severe episodes in some patients.[17] Several studies have reported that some children with AHC may require intensive care for breathing problems associated with whole-body

attacks and severe seizures, which are the main life-threatening symptoms associated with AHC.[17] A report from a large European study also mentioned 7 deaths due to severe plegic attacks or epileptic seizures.[14] These reports confirm that some patients with AHC have a severe clinical course. In our study, 8 of the 33 patients with AHC experienced stepwise or abrupt permanent severe motor deterioration, and none of these 8 patients showed any sign of recovery. Fever or status epilepticus could be a factor in this severe deterioration.

We investigated the severe motor deterioration in patients with AHC. We suspect that a genetic factor could be related to severe deterioration. Although severe motor deterioration was not observed among patients with the D801N mutation, it was observed in 5 of the 12 patients with an E815K mutation and 3 of the 11 patients with other specific mutations. We should be aware of the possibility of severe motor deterioration in patients, particularly among those with E815K and G755A/S mutations.

Previous studies have shown that patients with early-onset AHC fared the poorest in terms of development.[15,16] One reason for the correlation with early-onset and poor development could be that the E815K mutation is associated with a severe phenotype of AHC.

Patients with AHC who experience severe deterioration do not recover, which is similar to the outcome for patients with DYT12 who experience fixed dystonic symptoms. The difference in clinical symptoms between patients with AHC and DYT12 is probably because of differences in the position of the *ATP1A3* mutations or amino acid sequence changes, which could influence the structure, function, and protein expression of the Na^+/K^+-ATPase transporting pump. Mutations in *ATP1A3* can be clearly differentiated for AHC and DYT12,[6–10] but they could be viewed as an allelic disorder or as different aspects on a continuum of a single disease.[11] It is not yet clear why these 2 disorders are clinically different. AHC may be a severe manifestation, whereas DYT12 may be a milder type. Differences in *ATP1A3* mutations influence the function of Na^+/K^+-ATPase, and an intermediate phenotype must exist. The D923N mutation, which has already been reported as causing DYT12,[18,19] could be a mild form of AHC. In our study, the G-3-09 patient who had a D923N mutation showed later onset, normal cognitive function, frequent dystonia, and dysarthria. This patient could have an intermediate form of the disorder between AHC and DYT12.[20,21]

Most causative *ATP1A3* mutations lie within conserved domains or in the transmembrane region of the Na^+/K^+-ATPase enzyme protein.[6–8] The amount of the enzyme remains stable, but enzyme activity is reduced with both E815K and D801N mutations.[6] At a molecular level, the reason for the E815K mutation causing more severe symptoms is unclear.[22–25] E815K and G755A/S mutations could be responsible for the more severe subtypes of AHC because both E815 and G755 are predicted to be located in the cytoplasmic domains adjacent to the membrane.[6] The reason for the G755A/S mutations resulting in a more severe phenotype than the G755C mutation may be explained by the same molecular mechanism responsible for the relationship between D801Y in DYT12 and D801N in AHC. Further investigations of the function of Na^+/K^+-ATPase harboring *ATP1A3* mutations causing AHC or DYT12 should be performed to elucidate the mechanism of these disorders and develop proper treatments.

In patients with AHC, flunarizine administration is recommended,[26] because it has been reported to be effective in reducing the frequency and intensity of plegic attacks.[26,27] However, it is not known whether flunarizine protects patients with AHC from manifestations of permanent severe motor deterioration. In this study, flunarizine may have had a protective effect on severe motor deterioration. The genotype could also affect the decline in motor function on flunarizine discontinuation. Although the mechanism of flunarizine efficacy is not fully understood, it blocks calcium and sodium currents in cultured rat cortical neurons.[28] Flunarizine had been discontinued in some patients because 1) it had not been shown to reduce the frequency or duration of recurrent flaccid types of hemiplegic attacks, or 2) approval for flunarizine was withdrawn in Japan by the Ministry of Health and Welfare in 1999.[29] Since then, it has not been possible to prescribe flunarizine in Japan. Therefore, families of patients with AHC have to import flunarizine from foreign countries. None of the patients with severe deteriorations recovered even when flunarizine was readministered after their collapse. It is uncertain whether these patients would have experienced severe deterioration if they had continued flunarizine therapy.

Although there is no gold standard treatment for patients with AHC, extensive care, e.g., administration of flunarizine, anticonvulsants, immediate treatments for status epilepticus or apnea attacks, is essential. This is even more important for patients with substantial severe motor deterioration who have E815K and other mutations and have discontinued flunarizine therapy. We recommend that all patients with AHC, regardless of genotype, should not discontinue flunarizine administration even if this does not show any obvious short-term effectiveness against recurrent hemiplegic attacks. Because the number of patients with AHC in this study was small, a global prospective study with a larger population is necessary to confirm the protective effect of flunarizine.

In this study, we observed that the E815K 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 can be associated with abrupt or stepwise severe deterioration, particularly among patients with an E815K mutation. Genotype–phenotype correlations in AHC should be further explored in a global study.

AUTHOR CONTRIBUTIONS

M. Sasaki, A.I., Y.S., S. Tsuji, and S. Hirose designed the study, wrote the report, performed the literature search, and created the figures. A.I., N.M., K.I., and S. Hirose performed the Sanger sequencing and data analyses for the de novo single-nucleotide polymorphisms. M. Sasaki, A.I., and B.Z. performed the statistical analyses. M. Sasaki, Y.S., S. Takada, A.A., Y.T., H.A., S.Y., T.O., Y. Oda, H. I., S. Hirabayashi, A.Y., H.K., S.K., M. Shimono, S.N., M. Suzuki, T.Y., Y. Oyazato, S. Tsuneishi, S.O., K.Y., S.D., T.A., N.K., R.K., T.I., and H.O. obtained samples from patients and clinical data.

ACKNOWLEDGMENT

The authors thank all the members of the Japanese AHC Family Association and all the patients who participated in the study for their cooperation and Minako Yonetani and Akiyo Hamachi for technical assistance.

STUDY FUNDING

Supported in part by Grants-in-Aid for Scientific Research on Innovative Areas (25129708 to S. Hirose) and "Genome Science" from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan (221S0002, to A.I. and S. Hirose); MEXT-supported Program for the Strategic Research Foundation at Private Universities 2013-2017 (A.I. and S.Hirose); Grants-in-Aid for Scientific Research (A) (24249060, to S. Hirose), Challenging Exploratory Research (25670481, to S. Hirose), Young Scientists (B) (23791201, to A.I.), and Bilateral Joint Research Projects (to S. Hirose) from the Japan Society for the Promotion of Science (JSPS); a grant from the Adaptable and Seamless Technology Transfer Program through Target-driven R&D (A-STEP) Exploratory Research, Japan Science and Technology Agency (JSP); Grants-in-aid for the Research on Measures for Intractable Diseases (No. H23-Nanji-Ippan-78 and H25-Nnjitou-Ippan-9 to S.Hirose); Research Grants (21B-5, 24-7, to M. Sasaki, Y.S., and S. Hirose) for Nervous and Mental Disorders; Health and Labor Science Research Grants (21210301 and KB220001, to S. Hirose) and a Grant-in-Aid for Research on Measures for Intractable Diseases (No. H22-Nanji-Ippan-49 to S. Hirose) from the Ministry of Health, Labor and Welfare of Japan; a Research Grant from the Japan Foundation for Pediatric Research (to A.I.); a Research Grant from the Japan Epilepsy Research Foundation (to A.I.); a Research Grant from the Kaibara Morikazu Medical Science Promotion Foundation (to A.I.); the Joint Usage/Research Program of Medical Research Institute, Tokyo Medical and Dental University (S. Hirose); Grants from the Mitsubishi Foundation (S. Hirose) and Takeda Scientific Foundation (S. Hirose); "Central Research Institute for the Molecular Pathomechanisms of Epilepsy of Fukuoka University" and Recommended Projects of Fukuoka University (117016, to S. Hirose). The sponsors had no role in the design of the study, data collection and analysis, decision to publish, or in the preparation of the manuscript.

DISCLOSURE

The authors report no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

Received May 24, 2013. Accepted in final form September 12, 2013.

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Intermediate Form Between Alternating Hemiplegia of Childhood and Rapid-Onset Dystonia–Parkinsonism

In 2012, the Na^+/K^+-transporting ATPase subunit alpha-3 (*ATP1A3*) gene was confirmed as the causative gene in alternating hemiplegia of childhood (AHC).[1-3] In addition, this gene causes rapid-onset dystonia–parkinsonism (RDP/DYT12).[4] AHC occurs within 18 months of age, with symptoms of episodic hemiplegia and abnormal ocular movement, accompanied by developmental delay and cognitive deficit.[2] DYT12 usually manifests itself among teenagers to young adults as abrupt-onset dystonia with prominent bulbar involvement that continues throughout life.[4] Although these 2 disorders are believed to represent one spectrum,[1-3,5] no intermediate form between AHC and DYT12 has been reported yet.

An 8-year-old Japanese boy born to healthy parents at full term after an uneventful pregnancy had a normal developmental history. At 2 years and 0 months, he was suddenly unable to ambulate without altered consciousness. Flaccid paralysis was observed on the left side of his body. He improved gradually and recovered completely within 2 weeks. Subsequently, he experienced frequent right or left hemiplegic attacks of the dystonic type that lasted from a few hours to a few weeks. Fever and emotional excitement were the trigger factors. He was referred to our hospital at 4 years of age. He exhibited normal intelligence, dysarthria, drooling, ataxic gait, general mild hypotonia, and clumsiness. However, brain MRI and EEG findings were normal. Blood and urine metabolic tests were negative. He was diagnosed with atypical AHC. At 8 years of age, his episodic dystonic type of hemiplegic attacks continued. However, general mild hypotonia, ataxic gait, and dysarthria were still observed between dystonic attacks but were not progressive. *ATP1A3* analysis revealed a de novo heterozygous missense mutation, c.2767G>A, resulting in p.Asp923Asn (D923N).

The present case did not satisfy the clinical criteria of AHC[2] or DYT12[4] completely (Table 1). The D923N mutation of *ATP1A3* was reported previously in 6 cases.[6-8] Case 1 was a 20-year-old patient with typical DYT12,[6] and case 2 was a child whose symptom was abrupt onset of dystonia at 4 years of age.[7] Cases 3 to 6 were members of a dominant family[8]; only 1 of these 4 cases was diagnosed with typical AHC; the others revealed atypical AHC because the age of onset of the hemiplegic attacks was >2 years.

We consider that above-mentioned atypical AHC cases, including the present case, to be intermediate forms between AHC and DYT12. These cases provide evidence that AHC and DYT12 are one spectrum of disorders caused by *ATP1A3* abnormalities.[5] The common symptom in *ATP1A3*-related disease is a paroxysmal movement disorder, particularly dystonia. The age of onset and primary symptoms of typical AHC and DYT12 are considerably different; these differences may be caused by the varying activity and expression of Na^+/K^+ ATPase. Unlike the other mutations observed in AHC, the D923N mutation does not downregulate Na^+/K^+ ATPase.[1] Therefore, this can explain why the D923N mutation causes an intermediate form between AHC and DYT12. Because the D923N mutation causes typical DYT12,[6] it is suggested that veiled modifier genes or epigenetic factors affect Na^+/K^+ ATPase functions, resulting in expression of the various phenotypes. ■

Acknowledgments: We thank all the members of the patient's family for their helpful cooperation in this study. We also thank Minako Yonetani and Akiyo Hamachi for their excellent technical assistance.

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Funding agencies: This work was supported in part by a Grant-in-Aid for Scientific Research on Innovative Areas "Genome Science" from the Ministry of Education, Culture, Sports, Science and Technology of Japan (#221S0002); a Grant-in-Aid for Scientific Research (A) (#21249062, to Shinichi Hirose); a Grant-in-Aid for Challenging Exploratory Research (#23659529, to Shinichi Hirose); a Grant-in-Aid for Young Scientists (B) (#23791201, to Atsushi Ishii) from the Japan Society for the Promotion of Science (JSPS); grants from the Adaptable and Seamless Technology *Transfer Program through Target-driven R&D* (A-STEP) Exploratory Research, Japan Science and Technology Agency (JSP); a Research Grant (#21B-5 and #24-7, to Masayuki Sasaki, Yoshiaki Saito, and Shinichi Hirose) for Nervous and Mental Disorders from the Ministry of Health, Labor and Welfare of Japan, "Central Research Institute for the Molecular Pathomechanisms of Epilepsy of Fukuoka University," Recommended Projects of Fukuoka University (#117016); a research grant from the Japan Foundation for Pediatric Research (to Atsushi Ishii); a research grant from the Japan Epilepsy Research Foundation (to Atsushi Ishii); and a research grant from the Kaibara Morikazu Medical Science Promotion Foundation (to Atsushi Ishii). The funders had no role in the study design, data collection and analysis, decision to publish, or manuscript preparation.

Relevant conflicts of interest/financial disclosures: Nothing to report. Full financial disclosures and author roles may be found in the online version of this article.

Received: 9 March 2013; **Revised:** 17 June 2013;
Accepted: 4 August 2013
Published online 30 September 2013 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/mds.25659