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.

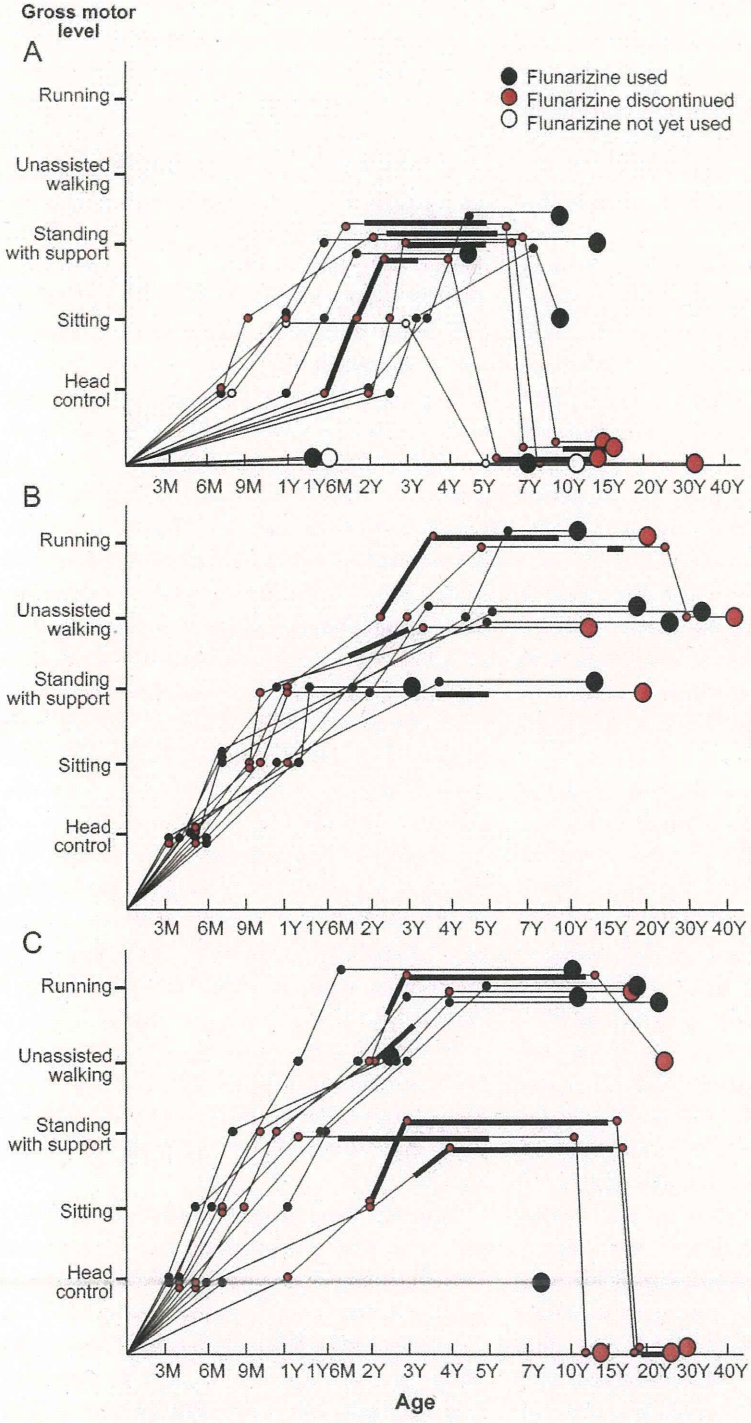
In group 3 (other mutations), the onset time was during the neonatal period in only 2 of the 11 patients. Most patients in this group showed slight delays in early development, and 7 of the 11 patients were able to walk unassisted in the interictal period between recurrent hemiplegic attacks. Six of 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.

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DISCLOSURE

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REFERENCES

1. Anderman F, Aicaridi J, Vigevano F, eds. Alternating Hemiplegia of Childhood. New York: Raven Press; 1995.
2. Bourgeois M, Aicardi J, Goutières F. Alternating hemiplegia of childhood. J Pediatr 1993;122:673–679.
3. Sweney MT, Silver K, Gerard-Blanluet M, et al. Alternating hemiplegia of childhood: early characteristics and evolution of a neurodevelopmental syndrome. Pediatrics 2009;123:e5345–e5341.
4. Tenney JR, Schapiro MB. Child neurology: alternating hemiplegia of childhood. Neurology 2010;74:e57–e59.
5. Swoboda KJ, Kanavakis E, Xaidara A, et al. Alternating hemiplegia of childhood or familial hemiplegic migraine? A novel ATP1A2 mutation. Ann Neurol 2004;55:884–887.
6. Heinzen EL, Swoboda KJ, Hitomi Y, et al. De novo mutations in *ATP1A3* cause alternating hemiplegia of childhood. Nat Genet 2012;44:1030–1034.
7. Rosewich H, Thiele H, Ohlenbusch A, et al. Heterozygous de-novo mutations in *ATP1A3* in patients with alternating hemiplegia of childhood: a whole-exome sequencing gene-identification study. Lancet Neurol 2012;11:764–773.
8. Ishii A, Saito Y, Mitsui J, et al. Identification of *ATP1A3* mutations by exome sequencing as the cause of alternating hemiplegia of childhood in Japanese patients. PLoS One 2013;8:e56120.
9. de Carvalho Aguiar P, Sweadner KJ, Penniston JT, et al. Mutations in the Na^+/K^+-ATPase α3 gene *ATP1A3* are associated with rapid-onset dystonia parkinsonism. Neuron 2004;43:169–175.
10. Brashear A, Dobyns WB, de Carvalho Aguiar P, et al. The phenotypic spectrum of rapid-onset dystonia-parkinsonism (RDP) and mutations in the *ATP1A3* gene. Brain 2007; 130:828–835.
11. Ozelius LJ. Clinical spectrum of disease associated with *ATP1A3* mutations. Lancet Neurol 2012;11:741–743.
12. Sakuragawa N. Alternating hemiplegia in childhood: 23 cases in Japan. Brain Dev 1992;14:283–288.
13. NevŠimalová S, Dittrich J, Havlová M, et al. Alternating hemiplegia in childhood; a cross-sectional study. Brain Dev 1994;16:189–194.
14. Panagiotakaki E, Gobbi G, Neville B, et al. Evidence of a non-progressive course of alternating hemiplegia of childhood: study of a large cohort of children and adults. Brain 2010;133:3598–3610.
15. Mikati MA, Kramer U, Zupanc ML, Shanahan RJ. Alternating hemiplegia of childhood: clinical manifestations and long-term outcome. Pediatr Neurol 2000;23:134–141.
16. Saito Y, Inui T, Sakakibara T, Sugai K, Sakuma H, Sasaki M. Evolution of hemiplegic attacks and epileptic seizures in alternating hemiplegia of childhood. Epilepsy Res 2010;90:248–258.
17. Neville BG, Ninan M. The treatment and management of alternating hemiplegia of childhood. Dev Med Child Neurol 2007;49:777–780.
18. Roubergue A, Roze E, Vuillaumier-Barrot S, et al. The multiple faces of the *ATP1A3*-related dystonic movement disorder. Mov Disord 2013;28:1457–1459.
19. Anselm IA, Sweadner KJ, Gollamudi S, Ozelius LJ, Darras BT. Rapid-onset dystonia-parkinsonism in a child with a novel *ATP1A3* gene mutation. Neurology 2009;73:400–401.
20. Brashear A, Mink JW, Hill DF, et al. *APT1A3* mutation in infants: a new rapid-onset dystonia-Parkinsonism phenotype characterized by motor delay and ataxia. Dev Med Child Neurol 2012;54:1065–1067.
21. Sasaki M, Ishii A, Saito Y, Hirose S. Intermediate form between alternating hemiplegia of childhood and rapid-onset dystonia–parkinsonism. Mov Disord Epub 2013 Sep 30.
22. Morth JP, Pedersen BP, Toustrup-Jensen MS, et al. Crystal structure of the sodium-potassium pump. Nature 2007; 450:1043–1049.

23. Ogawa H, Shinoda T, Cornelius F, Toyoshima C. Crystal structure of the sodium-potassium pump (Na^+/K^+ ATPase) with bound potassium and ouabain. Proc Natl Acad Sci USA 2009;106:13742–13747.
24. Shinoda T, Ogawa H, Cornelius F, Toyoshima C. Crystal structure of the sodium-potassium pump at 2.4 Å resolution. Nature 2009;459:446–450.
25. Poulsen H, Khandelia H, Morth JP, et al. Neurological disease mutations compromise a C-terminal ion pathway in the Na^+/K^+ ATPase. Nature 2010;467:99–102.
26. Casear P, Azou M. Flunarizine in alternating hemiplegia in childhood. Lancet 1984;2:579.
27. Silver K, Andermann F. Alternating hemiplegia of childhood: a study of 10 patients and results of flunarizine treatment. Neurology 1993;43:36–41.
28. Ye Q, Yan L, Xue L, et al. Flunarizine blocks voltage-gated Na^+ and Ca^{2+} currents in cultured rat cortical neurons: a possible locus of action in the prevention of migraine. Neurosci Lett 2011;487:394–399.
29. Sasaki M, Sakuragawa N, Osawa M. Long-term effect of flunarizine on patients with alternating hemiplegia of childhood in Japan. Brain Dev 2001;23:303–305.

Evaluation of *SLC20A2* mutations that cause idiopathic basal ganglia calcification in Japan

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Evaluation of *SLC20A2* mutations that cause idiopathic basal ganglia calcification in Japan

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ABSTRACT

Objective: To investigate the clinical, genetic, and neuroradiologic presentations of idiopathic basal ganglia calcification (IBGC) in a nationwide study in Japan.

Methods: We documented clinical and neuroimaging data of a total of 69 subjects including 23 subjects from 10 families and 46 subjects in sporadic cases of IBGC in Japan. Mutational analysis of *SLC20A2* was performed.

Results: Six new mutations in *SLC20A2* were found in patients with IBGC: 4 missense mutations, 1 nonsense mutation, and 1 frameshift mutation. Four of them were familial cases and 2 were sporadic cases in our survey. The frequency of families with mutations in *SLC20A2* in Japan was 50%, which was as high as in a previous report on other regions. The clinical features varied widely among the patients with *SLC20A2* mutations. However, 2 distinct families have the same mutation of S637R in *SLC20A2* and they have similar characteristics in the clinical course, symptoms, neurologic findings, and neuroimaging. In our study, all the patients with *SLC20A2* mutations showed calcification. In familial cases, there were symptomatic and asymptomatic patients in the same family.

Conclusion: *SLC20A2* mutations are a major cause of familial IBGC in Japan. The members in the families with the same mutation had similar patterns of calcification in the brain and the affected members showed similar clinical manifestations. ***Neurology*® 2014;82:705–712**

GLOSSARY

DNTC = diffuse neurofibrillary tangles with calcification; **FIBGC** = familial idiopathic basal ganglia calcification; **IBGC** = idiopathic basal ganglia calcification; **MMSE** = Mini-Mental State Examination; **PDGF** = platelet-derived growth factor; **PDGFRB** = platelet-derived growth factor receptor-β; **Pi** = inorganic phosphate; **PiB** = Pittsburgh compound B; **PiT** = type III sodium-dependent phosphate transporter; **PKC** = paroxysmal kinesigenic choreoathetosis.

Idiopathic basal ganglia calcification (IBGC), also known as Fahr disease, is thought to be a rare neuropsychiatric disorder characterized by symmetrical calcification in the basal ganglia and other brain regions. Clinical manifestations range widely from asymptomatic to variable symptoms including headaches, psychosis, and dementia.[1] The diagnosis of IBGC generally relies on the visualization of bilateral calcification mainly in the basal ganglia by neuroimaging and the absence of metabolic, infectious, toxic, or traumatic causes.[2,3]

The mode of inheritance of familial IBGC (FIBGC) has been thought to be autosomal dominant and, to date, 4 responsible chromosomal regions have been identified, namely 14q (IBGC1), 2q37 (IBGC2), 8p11.21 (IBGC3), and 5q32 (IBGC4).[3–14] The causative gene at the IBGC3 locus was identified as *SLC20A2* encoding type III sodium-dependent phosphate transporter 2 (PiT-2). Screening of a large series of patients with IBGC revealed that mutations in *SLC20A2* are a major cause of FIBGC[10]; moreover, other mutations in *SLC20A2* have recently been reported in China and Brazil.[11–13] The mutations of *PDGFRB* encoding platelet-derived growth factor

Supplemental data at www.neurology.org

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(PDGF) receptor-β (PDGFRB) and *PDGFB* have recently been reported to cause calcification in the brain.[14,15]

We have collected clinical information of patients with IBGC in a nationwide survey in Japan. Here, on the basis of a mutational analysis of *SLC20A2*, we aim to establish the molecular epidemiology of IBGC3 and evaluate clinically and genetically *SLC20A2* mutations in Japan.

METHODS Subjects and samples. We collected clinical information on patients with IBGC in a nationwide study. The criteria for the selection of patients in the initial survey were as follows: 1) conspicuous calcification is observed in the basal ganglia and/or dentate nucleus by CT scan; 2) calcification is bilateral and symmetrical; and 3) idiopathic (absence of biochemical abnormalities, and an infectious, toxic, or traumatic cause).[2,3] Neurologists enrolled patients in the survey. They examined the medical charts and performed the neurologic examinations again if necessary. The survey was approved by the Ethics Committee of the Gifu University Graduate School of Medicine. During the survey, some patients were found to have hypoparathyroidism, Aicardi-Goutières syndrome, and Cockayne syndrome, and these patients were excluded. For the genetic study, a total of 69 subjects from 41 hospitals provided written informed consent and were enrolled in the project. Of these patients, 46 came from families with a single affected member, and the other 23 came from 10 families with multiple affected members. We defined the former as sporadic patients and the latter as familial patients. The patients' mean age ± SD was 41.3 ± 23.6 years at registration. The patients comprised 32 males and 37 females.

Standard protocol approvals, registrations, and patient consents. All experiments on human DNA were approved by the Ethics Committees of both Gifu University and the University of Tokyo. After written informed consent was obtained, peripheral blood samples were collected.

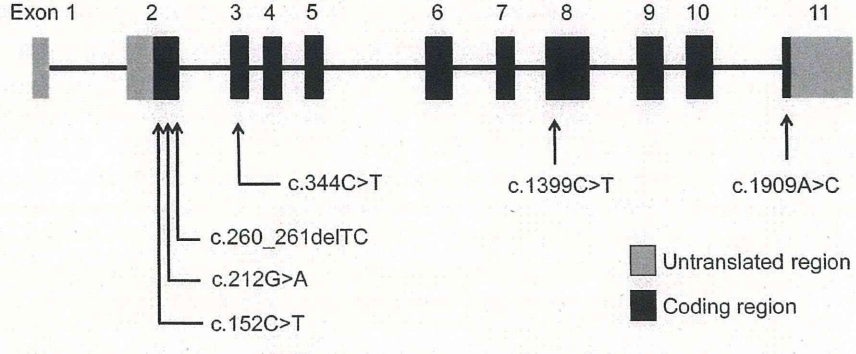
Mutational analysis. Genomic DNA was extracted from the whole blood samples. *SLC20A2* analysis was performed by Sanger sequencing of all coding regions, as described in detail in e-Methods and table e-1, A and B, on the *Neurology*® Web site at www.neurology.org. The pathologic potential of the identified variants was predicted using PolyPhen-2 (http://genetics.bwh.harvard.edu/pph/).[16]

RESULTS Mutational analysis. We screened a total of 69 subjects including 23 subjects from 10 families in which multiple affected subjects were observed and 46 subjects in sporadic cases, all of whom were Japanese. Six new mutations in *SLC20A2* were found: 4 missense mutations, 1 nonsense mutation, and 1 frameshift mutation (figure 1). Electropherograms showed the individual heterozygous mutations (figure e-1). None of them were present in an in-house exome sequencing data set (358 Japanese control subjects), dbSNP 137 (www.ncbi.nlm.nih.gov/snp/), or the National Heart, Lung, and Blood Institute "Grand Opportunity" Exome Sequencing Project (ESP6500SI-V2). In silico analysis predicted deleterious consequences, as determined from the residue changes in figures 1 and e-1. When confined to the FIBGC patients, 5 of the 10 families (50.0%) showed mutations in *SLC20A2*. In contrast, 2 of the 46 patients (4.3%) with sporadic IBGC carried mutations in *SLC20A2* in this study.

Clinical manifestations. The clinical manifestations are summarized in table 1. A positive family history of IBGC was present in 5 families. Families 1 and 2 had the same mutation.

Familial cases. ***Case 1 (in family 1).*** The proband in family 1 was a 64-year-old woman who had dysarthria and gait disturbance for 5 years. She showed no dementia. Her neurologic examination revealed dysarthria, small steppage gait, rigidity at bilateral wrist joints, bradykinesia, and a pyramidal sign. Her CT images revealed severe calcification at the bilateral globus pallidus, caudate nuclei, thalamus, subcortical white matter, and dentate nuclei (figure 2C). Her son's CT showed similar brain calcification (figure 2D), although he was clinically asymptomatic. His DNA study revealed the

Figure 1 Schematic representation of causative mutations in *SLC20A2* in idiopathic basal ganglia calcification



Six new causative mutations in exon 2 (c.152C>T, c.212 G>A, c.260_261delTC), exon 3 (c.344C>T), exon 8 (c.1399C>T), and exon 11 (c.1909A>C) were found in this study.