4. Discussion

Molecular elucidation of LS at the DNA level is challenging. LS has been associated with a variety of genes in either mitochondrial or nuclear encoded DNA [3]. Surprisingly, we could reveal mutations in 61% of LS patients (11/18 individuals).

We disclosed 7 patients with mDNA mutations. From mitochondrial *ND1*, we identified an m3697G>A mutation in 2 unrelated patients, which has been reported previously in association with mitochondrial myopathy, encephalopathy, lactic acidosis, stroke-like episodes (MELAS) [9] and Leber's hereditary optic neuropathy (LHON) [10]. To our knowledge, this is the first report of the m3697G>A/*ND1* gene mutation causing Leigh syndrome. The heteroplasmy rate is reportedly 80% in patients with MELAS (skeletal muscle) and was 56% with LOHN [9,10]. A high mutation load (100%), found in the blood of Patients 1 and 2 may be associated to severe phenotype in our patients [11]. Low level of m3697G>A mutation (~40%) was found in the blood from an asymptomatic mother of Patient 1 (Suppl. Figs. 3 and 4).

For ND3, we found a mutation of m10158T>C with 90% of heteroplasmic rate in one patient showing an early onset and very rapid progress. Severe clinical course and high mutant loads are consistent with reported cases with rapid progression and lethal consequences at early childhood [12]. A mutation of m10158T>C was not detected in the mother of Patient 3 in several tissues examined.

We found one patient with ND5 mutation, m13513G>A which has been described as causing MELAS, LS or overlapping features of the two syndromes [13–15]. We also found one LS patient with m14459G>A/*ND6* mutation that was reported in patients with LHON, dystonia [16] and LS [17]. So far, the phenotype of these two patients is LS without MELAS, LHON.

We found two patients with *ATPase6* mDNA mutations, m8993T>G and T>C, that are frequently reported in the literature [8]. A patient with a T>G mutation usually exhibits earlier onset and more rapid progression compared to T>C mutation at m8993 that was compatible with our patients (Table 1).

We found 4 patients carrying nuclear encoded gene mutations. SURF1 deficiency is the most frequent cause of LS with complex IV (cytochrome C oxidase) deficiency [7]. We identified 3 patients with the *SURF1* mutations [18]. Pyruvate dehydrogenase deficiency (PDH) is a common cause of primary congenital lactic acidosis. The biochemical features of PDH deficiency is elevated blood lactate and pyruvate levels with a normal lactate/pyruvate ratio [19]. According to the genetic screening flowchart for Leigh syndrome (Suppl. Fig. 1), we confirmed 1 patient with a hemizygous mutation in the *PDHA1* gene with 7 sets of primers.

Recently, new drugs such as EPI-743 have been shown to improve neurological and neuromuscular symptoms in LS [20,21]. Rapid genetic confirmation of mitochondrial disease may help initiate such treatment early. Next gene sequencing is revealing a wide range of dual mutations both mitochondrial and nuclear gene from patients with mitochondrial disorders [22–24]. However, it is costly and time consuming. Aiming to elucidate genetic basis of LS patients, we screened with our limited set of primers. Surprisingly, it allowed us confirmation for more than half of the patients. Therefore, this method appears to be efficient as a primary genetic screening. Our data also implicates that LS consisted of few "common" causative genes and a large number of "rare" genes. We are now undertaking whole mDNA and exome sequencing for negative cases of this method [22–24]. These data, together with increasing data of mutations, would help us improve our screening method.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ymgmr.2014.02.006.

Conflict of interest statement

We have no conflict of interest to disclose.

Acknowledgments

This work was supported in part by a grant of the Innovative Cell Biology by Innovative Technology (Cell Innovation Program) from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japan, by Grants-in-Aid of the Research on Intractable Diseases (Mitochondrial Disorder) from the Ministry of Health, Labour and Welfare of Japan (H23-N-I-016), and by Kawano Masanori Memorial Public Interest Incorporated Foundation for Promotion of Pediatrics (24-12).

Table 1
Genetically determined Leigh syndrome in our institution (2005–2012).

Patient	1	2	3	4	5	6	7	8	9	10	11
Age, gender	7 y, M	10 y, F	9 m, F	7 y, M	11 y, M	1 y†, M	2 y, M	4 y, F	9 y, M	25 y†, M	17 y, M
Type of gene	Mito	Mito	Mito	Mito	Mito	Mito	Mito	Nuclear	Nuclear	Nuclear	Nuclear
Gene	*ND1*	*ND1*	*ND3*	*ND5*	*ND6*	*ATPase6*	*ATPase6*	*SURF1*	*SURF1*	*SURF1*	*PDHA1*
Complex	I	I	I	I	I	V	V	IV	IV	IV	
Mutations	m3697G>A (p.G131S) Homo (b)	m3697G>A (p.G131S) Homo (b,s,h,n)	m10158T>C (p.S34P) Hetero (90%) (b)	m13513G>A (p.D393N) Hetero (50%) (b)	m14459G>A (p.A71V) Homo (b)	m8993T>G (p.L156R) Homo (b)	m8993T>C (p.L156P) Homo (b)	c.49+1G>T c.752–753delAG	c.743 C> A p.A248D c.743C> A p.A248D	c.574C>T p.R192 W c.743C>A p.A248D	c.121T>C p.C41R
Consanguinity	N	N	N	N	N	N	N	N	Y	N	N
Inheritance	Maternal* hetero:40%	N.A.	De novo	N.A.	N.A.	N.A.	N.A.	Maternal/ paternal	Maternal/ paternal	N.A.	N.A.
Age at onset	3 y 9 m	3 y 0 m	0 y 5 m	1 y 6 m	2 y 0 m	6 m	1 y 0 m	1 y 7 m	1 y 9 m	2 y	1 y 0 m
Initial Symptoms	Hypertonia Walk regre	Ataxic gait Walk regre Tremor	Hypotonia Strabismus	Dev. delay	Fever → lethargy	Dev. delay/ seizure Hypotonia/ nystagmus	Fever → lethargy	Ataxic gait	Ataxic gait	Dev. delay Ataxia	Dev. delay
Status	Walk Normal class	Wheelchair Special class	Tracheo Mech. venti	Walk	Wheelchair Normal class	(Respiratory failure)	No sitting	Tracheo Mech. venti	Tracheo Mech. venti	(Respiratory failure)	Walk Special school
RC enzymes ↓	I, IV (m)	I, III, IV (m)	I (f)	Normal (m/f)	I, III (m)	I, IV (m)	N.A.	N.A.	IV (f)	IV (m)	N.A.
Morphological findings in muscle	No RRF	No RRF	N.A.	No RRF	RRF	N.A.	N.A.	N.A.	N.A.	RRF	N.A.

MRI											
Basal ganglia hyperintensities	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
Brainstem hyperintensities	N	Y	Y	N	N	N	Y	Y	Y	Y	N
Cerebellar atrophy	N	N	N	Y	N	N	N	N	N	Y	Y
Symptoms											
Dysmorphisms	N	N	N	N	N	N	N	Y	Y	N	N
Developmental delay	N	N	Y	Y	N	N	Y	Y	Y	Y	N
Regression	Y	Y	Y	N	N	Y	Y	Y	Y	Y	N
Feeding problems	N	N	Y	N	N	Y	N	N	N	N	N
Ptosis	N	N	N	N	N	N	N	Y	N	N	N
Ophthalmople	N	N	Y	N	N	N	N	Y	N	Y	N
Pyramidal symptoms	Y	Y	Y	Y	Y	N	N	Y	Y	N	Y
Extrapyramidal symptoms	Y	Y	Y	Y	N	Y	N	Y	Y	N	Y
Dystonia	Y	Y	Y	N	N	N	N	Y	Y	N	Y
Hypotonia	N	N	Y	Y	N	Y	Y	Y	Y	N	Y
Ataxia	Y	Y	Y	Y	N	N	N	Y	Y	Y	Y
Neuropathy	N	N	N	N	N	N	N	Y	Y	Y	Y
Others				WPW syndrome		West syndrome					Nystagmus

y: year, m: month, M: male, F: female, mito: mitochondria, Complex: complex in oxidative phosphorylation, b: blood, s: saliva, h: hair, n: nail, RC: respiratory chain, m: muscle, f: fibroblast, RRF: ragged red fibers, N.A.: not analyzed/not determined, N: no, negative, Y: yes, positive, regre: regression, Dev. delay: Developmental delay, Mech.venti: Mechanically ventilated, Ophthalmople: Ophthalmoplegia, *: asymptomatic.

References

[1] D. Leigh, Subacute necrotizing encephalomyelopathy in an infant, J. Neurol. Neurosurg. Psychiatry 14 (1951) 216–221.
[2] S. Rahman, R.B. Blok, H.H. Dahl, D.M. Danks, D.M. Kirby, C.W. Chow, J. Christodoulou, D.R. Thorburn, Leigh syndrome: clinical features and biochemical and DNA abnormalities, Ann. Neurol. 39 (1996) 343–351.
[3] J. Finsterer, Leigh and Leigh-like syndrome in children and adults, Pediatr. Neurol. 39 (2008) 223–235.
[4] E. Fassone, S. Rahman, Complex I deficiency: clinical features, biochemistry and molecular genetics, J. Med. Genet. 49 (2012) 578–590.
[5] S. Koene, R.J. Rodenburg, M.S. van der Knaap, M.A. Willemsen, W. Sperl, V. Laugel, E. Ostergaard, M. Tarnopolsky, M.A. Martin, V. Nesbitt, J. Fletcher, S. Edvardson, V. Procaccio, A. Slama, L.P. van den Heuvel, J.A. Smeitink, Natural disease course and genotype–phenotype correlations in complex I deficiency caused by nuclear gene defects: what we learned from 130 cases, J. Inherit. Metab. Dis. 35 (2012) 737–747.
[6] Z. Zhu, J. Yao, T. Johns, K. Fu, I. De Bie, C. Macmillan, A.P. Cuthbert, R.F. Newbold, J. Wang, M. Chevrette, G.K. Brown, R.M. Brown, E.A. Shoubridge, SURF1, encoding a factor involved in the biogenesis of cytochrome c oxidase, is mutated in Leigh syndrome, Nat. Genet. 20 (1998) 337–343.
[7] Y. Wedatilake, R. Brown, R. McFarland, J. Yaplito-Lee, A.A. Morris, M. Champion, P.E. Jardine, A. Clarke, D.R. Thorburn, R.W. Taylor, J.M. Land, K. Forrest, A. Dobbie, L. Simmons, E.T. Aasheim, D. Ketteridge, D. Hanrahan, A. Chakrapani, G.K. Brown, S. Rahman, SURF1 deficiency: a multi-centre natural history study, Orphanet J. Rare Dis. 8 (2013) 96.
[8] M. Makino, S. Horai, Y. Goto, I. Nonaka, Mitochondrial DNA mutations in Leigh syndrome and their phylogenetic implications, J. Hum. Genet. 45 (2000) 69–75.
[9] D.M. Kirby, R. McFarland, A. Ohtake, C. Dunning, M.T. Ryan, C. Wilson, D. Ketteridge, D.M. Turnbull, D.R. Thorburn, R.W. Taylor, Mutations of the mitochondrial ND1 gene as a cause of MELAS, J. Med. Genet. 41 (2004) 784–789.
[10] L. Spruijt, H.J. Smeets, A. Hendrickx, M.W. Bettink-Remeijer, A. Maat-Kievit, K.C. Schoonderwoerd, W. Sluiter, I.F. de Coo, R.Q. Hintzen, A MELAS-associated ND1 mutation causing leber hereditary optic neuropathy and spastic dystonia, Arch. Neurol. 64 (2007) 890–893.
[11] A.L. Mitchell, J.L. Elson, N. Howell, R.W. Taylor, D.M. Turnbull, Sequence variation in mitochondrial complex I genes: mutation or polymorphism? J. Med. Genet. 43 (2006) 175–179.
[12] R. McFarland, D.M. Kirby, K.J. Fowler, A. Ohtake, M.T. Ryan, D.J. Amor, J.M. Fletcher, J.W. Dixon, F.A. Collins, D.M. Turnbull, R.W. Taylor, D.R. Thorburn, De novo mutations in the mitochondrial ND3 gene as a cause of infantile mitochondrial encephalopathy and complex I deficiency, Ann. Neurol. 55 (2004) 58–64.
[13] A. Sudo, S. Honzawa, I. Nonaka, Y. Goto, Leigh syndrome caused by mitochondrial DNA G13513A mutation: frequency and clinical features in Japan, J. Hum. Genet. 49 (2004) 92–96.
[14] M. Crimi, S. Galbiati, I. Moroni, A. Bordoni, M.P. Perini, E. Lamantea, M. Sciacco, M. Zeviani, I. Biunno, M. Moggio, G. Scarlato, G.P. Comi, A missense mutation in the mitochondrial ND5 gene associated with a Leigh–MELAS overlap syndrome, Neurology 60 (2003) 1857–1861.
[15] A. Brautbar, J. Wang, J.E. Abdenur, R.C. Chang, J.A. Thomas, T.A. Grebe, C. Lim, S.W. Weng, B.H. Graham, L.J. Wong, The mitochondrial 13513G>A mutation is associated with Leigh disease phenotypes independent of complex I deficiency in muscle, Mol. Genet. Metab. 94 (2008) 485–490.
[16] A. Gropman, T.J. Chen, C.L. Perng, D. Krasnewich, E. Chernoff, C. Tifft, L.J. Wong, Variable clinical manifestation of homoplasmic G14459A mitochondrial DNA mutation, Am. J. Med. Genet. A 124A (2004) 377–382.
[17] D.M. Kirby, S.G. Kahler, M.L. Freckmann, D. Reddihough, D.R. Thorburn, Leigh disease caused by the mitochondrial DNA G14459A mutation in unrelated families, Ann. Neurol. 48 (2000) 102–104.
[18] J. Tanigawa, K. Kaneko, M. Honda, H. Harashima, K. Murayama, T. Wada, K. Takano, M. Iai, S. Yamashita, H. Shimbo, N. Aida, A. Ohtake, H. Osaka, Two Japanese patients with Leigh syndrome caused by novel SURF1 mutations, Brain Dev. 34 (2012) 861–865.
[19] L. De Meirleir, Defects of pyruvate metabolism and the Krebs cycle, J. Child Neurol. 17 (Suppl. 3) (2002) 3S26-33 (discussion 3S33-24).
[20] A.A. Sadun, C.F. Chicani, F.N. Ross-Cisneros, P. Barboni, M. Thoolen, W.D. Shrader, K. Kubis, V. Carelli, G. Miller, Effect of EPI-743 on the clinical course of the mitochondrial disease Leber hereditary optic neuropathy, Arch. Neurol. 69 (2012) 331–338.
[21] D. Martinelli, M. Catteruccia, F. Piemonte, A. Pastore, G. Tozzi, C. Dionisi-Vici, G. Pontrelli, T. Corsetti, S. Livadiotti, V. Kheifets, A. Hinman, W.D. Shrader, M. Thoolen, M.B. Klein, E. Bertini, G. Miller, EPI-743 reverses the progression of the pediatric mitochondrial disease—genetically defined Leigh syndrome, Mol. Genet. Metab. 107 (2012) 383–388.
[22] D.S. Lieber, S.E. Calvo, K. Shanahan, N.G. Slate, S. Liu, S.G. Hershman, N.B. Gold, B.A. Chapman, D.R. Thorburn, G.T. Berry, J.D. Schmahmann, M.L. Borowsky, D.M. Mueller, K.B. Sims, V.K. Mootha, Targeted exome sequencing of suspected mitochondrial disorders, Neurology 80 (2013) 1762–1770.
[23] J.T. Dare, V. Vasta, J. Penn, N.T. Tran, S.H. Hahn, Targeted exome sequencing for mitochondrial disorders reveals high genetic heterogeneity, BMC Med. Genet. 14 (2013) 118–126.
[24] D.L. Dinwiddie, L.D. Smith, N.A. Miller, A.M. Atherton, E.G. Farrow, M.E. Strenk, S.E. Soden, C.J. Saunders, S.F. Kingsmore, Diagnosis of mitochondrial disorders by concomitant next-generation sequencing of the exome and mitochondrial genome, Genomics 102 (2013) 148–156.

Pediatric Neurology 50 (2014) 99–100

Contents lists available at ScienceDirect

Pediatric Neurology

journal homepage: www.elsevier.com/locate/pnu

Clinical Observations

A Three-Year-Old Boy With Glucose Transporter Type 1 Deficiency Syndrome Presenting With Episodic Ataxia

Akiko Ohshiro-Sasaki MD, Hiroko Shimbo, Kyoko Takano MD, PhD, Takahito Wada MD, PhD *, Hitoshi Osaka MD, PhD

Division of Neurology, Kanagawa Children's Medical Center, Yokohama, Japan

ABSTRACT

INTRODUCTION: Glucose transporter type 1 deficiency syndrome is a metabolic encephalopathy that results from impaired glucose transport into the brain as the result of a mutation of the *SLC2A1* gene. It has been recognized recently that these patients can present with a much broader clinical spectrum than previously thought. We describe a 3-year-old boy presenting with episodic ataxia. **CASE REPORT:** Our patient exhibited periodic abnormal eye movements, including opsoclonus, since he was 4 months of age. At 2 years of age, he experienced acute cerebellar ataxia after a vaccination. Since then, he has had periodic attacks of ataxic gait, repeated vomiting, and abnormal eye movement. He was diagnosed as having episodic ataxia type 2 because the administration of acetazolamide seemed effective. By 3 years and 10 months of age, he exhibited mild mental retardation and mild trunk ataxia. The attacks were more likely to occur when he was hungry. Molecular analysis revealed that the *SLC2A1* gene had a de novo mutation of heterozygous seven nucleotide insertion within exon 7, resulting in a frameshift. He has recently begun a modified Atkins diet; the frequency of attacks has been reduced, and his psychomotor and language skills have begun to develop. **DISCUSSION:** Glucose transporter type 1 deficiency syndrome should be considered in the differential diagnosis in children with episodic ataxia, even if acetazolamide is effective.

Keywords: glucose transporter type 1 deficiency syndrome, episodic ataxia, *SLC2A1*, cerebellar ataxia, intellectual disability, seizure, ketogenic diet

Pediatr Neurol 2014; 50: 99-100

Introduction

Glucose transporter type 1 deficiency syndrome (GLUT1-DS) is caused by a defect of the glucose transporter, Glut1, the fundamental vehicle that facilitates glucose entry into the brain and across the astrocyte membrane, which is coded by the *SLC2A1* gene. GLUT1-DS was first described in 1991 as a metabolic encephalopathy characterized by epileptic seizures, delayed development, ataxia, dystonia, and acquired microcephaly (classic GLUT1-DS).[1]

The laboratory hallmark of GLUT1-DS is a low cerebrospinal fluid (CSF) glucose concentration (<40 mg/dL) and a low ratio of CSF/blood glucose (<0.4), Recently, a broader spectrum of complex clinical presentations of GLUT1-DS has been recognized.[2] Neurological features may be divided into three symptom domains: seizure, movement disorders, and cognitive/behavioral disturbances.[3] Three different phenotypes of GLUT1-DS are defined; (1) classical (early <2 years of age and late >2 years of age), (2) nonclassical, and (3) GLUT1-DS with minimal symptoms.[4] Here, we describe a 3-year-old boy with GLUT1-DS who presented with episodic ataxia.

Case Report

This 3-year-old boy was the first child of healthy unrelated parents. He was born at 39 weeks of gestational age after an uneventful pregnancy and delivery. He had recurrent attacks of abnormal eye movement, including opsoclonus, at 4, 7, and 11 months of age. Epilepsy was initially suspected, but he did not receive antiepileptic treatment because the

Article History:
Received February 4, 2013; Accepted in final form September 1, 2013

* Communications should be addressed to: Dr. Takahito Wada; Mutsukawa 2-138-4; Minami-ku; Yokohama; Kanagawa 232-8555, Japan.
E-mail address: takahito.wada0001@me.com

0887-8994/$ - see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.pediatrneurol.2013.09.002

findings of an electroencephalography did not reveal epileptic discharges. He started walking independently at 18 months of age. When he was 2 years of age, he could not stand or walk for 2 weeks after receiving vaccinations for *Haemophilus influenzae* Type b, diphtheria, pertussis, and tetanus. He was diagnosed with acute cerebellar ataxia and recovered completely in a few days after hydration treatment. He was referred to our medical center at 2 years of age for the evaluation of his delayed development. He had no apparent neurological abnormalities, no pyramidal or extrapyramidal signs, and no cerebellar signs, such as ataxic gait or abnormal eye movement. He started to use meaningful words at 2 years of age, and his receptive language skills seemed better than his expressive language skills. He was diagnosed with a mild intellectual disability or delayed expressive language disorder.

Beginning at 2 years and 4 months of age, he had recurrent attacks of acute cerebellar ataxia that lasted for 2-3 hours, once or twice a month. The attacks consisted of three features: ataxic gait, repeated vomiting, and abnormal eye movement. Because oral acetazolamide treatment reduced the frequency of the attacks, episodic ataxia type 2 (EA2) was suspected. Magnetic resonance imaging of his brain did not show cerebellar atrophy or other abnormalities. When he was 3 years of age, his mother noticed that the attacks were more likely to occur when he was hungry, mostly in the early morning, and that his intermittent ataxic gait improved after having a meal, especially greasy food such as fried chicken. He had his first afebrile generalized tonic seizure at 3 years of age, and the findings of electroencephalography revealed no paroxysmal discharges.

At 3 years and 10 months of age, he was admitted for evaluation. His height, weight, and head circumference were 97 cm (−0.7 SD), 14.3 kg (−0.6 SD), and 47.7 cm (−1.6 SD), respectively, indicating acquired microcephaly. A neurological examination revealed mild truncal ataxia but no pyramidal or extrapyramidal signs. His developmental quotient was 47, indicating moderate intellectual disability. CSF glucose was 38 mg/dL and blood glucose was 102 mg/dL, with a CSF/blood ratio of 0.37. Levels of CSF lactate and pyruvate were normal (0.90 and 0.065 mmol/L, respectively). Thus, GLUT1-DS was suspected. Analysis of the DNA extracted from his blood revealed that *SLC2A1* has a de novo and novel heterozygous mutation of seven nucleotides insertion in exon 7, resulting in truncated protein [c.930_931 ins-GGATACC, p.Ile311 fs], and his diagnosis was confirmed.

He has recently begun a modified Atkins diet, eating five times per day. The frequency of his attacks is reducing, his psychomotor and language skills are improving, and he can now speak in multiple sentences and can concentrate.

Discussion

The phenotypic spectrum of GLUT1-DS appears to be more variable than previously recognized. Our patient presented with intermittent cerebellar ataxia as the initial manifestation, similar to EA2, with mild truncal ataxia, whereas dystonia, chorea, or epilepsy were not apparent and cognitive disturbance was mild to moderate. Only one patient has been reported to have had intermittent ataxia as the initial manifestation and used acetazolamide like our patient.[5] In some reports, authors describe patients with cerebellar ataxia, but most of their cerebellar ataxia is chronic, and most had other motor abnormalities, including abnormal gait, dystonia, chorea, cerebellar intention tremor, and myoclonus.[4]

It is important to consider that GLUT1-DS and other channelopathies, such as EA2 caused by mutations of the *CACNA1A* gene, share chronic and intermittent clinical features and responsiveness to acetazolamide.[6] It is possible that chronic neuroglycopenia may lead to developmental alternations in channel expression or function, causing abnormal neuronal excitability in GLUT1-DS.[7] Responsiveness to acetazolamide may be a key component in the diagnosis of GLUT1-DS.[8]

A relationship between the clinical severity and the specific type of *SLC2A1* mutation has been noted.[4] Considering that many patients with atypical or milder cases remain to be diagnosed, it seems too hasty to establish the genotype–phenotype correlations. As discussed in the paragraphs to follow, in addition to the type of mutation, the patient's dietary habit from infancy may affect the phenotype.

This finding provides a clue in the diagnosis of GLUT1-DS in terms of confirming the correlation between fluctuations of neurological symptoms and fasting. Some patients have characteristic dietary habits that prevent a deterioration of neurological functioning. Some patients eat meals every 2-3 hours and wake at night to eat sweets. Another patient preferred to be served honey at bedside before rising in the morning.[9]

A ketogenic diet is currently the treatment of choice for GLUT1-DS. A ketogenic diet markedly improves seizures, movement disorder, and head growth. Recently, a modified Atkins diet has also been used successfully in patients with GLUT1-DS.[10] Our patient has recently commenced dietary treatment on the basis of a modified Atkins diet. The frequency of attacks is reduced, and his psychomotor and language skills have begun developing, although longer observation is needed to confirm that this therapy is effective. We should consider GLUT1-DS as a differential diagnosis in pediatric patients with intermittent cerebellar ataxia such as EA2 or intermittent neurological phenotypes such as other channelopathies.

References

1. De Vivo DC, Trifiletti RR, Jacobson RI, Ronen GM, Behmand RA, Harik SI. Defective glucose transport across the blood-brain barrier as a cause of persistent hypoglycorrhachia, seizures, and developmental delay. *N Engl J Med*. 1991;325:703-709.
2. Tzadok M, Nissenkorn A, Porper K, et al. The many faces of Glut1 deficiency syndrome. *J Child Neurol*; 2013. (Epub ahead of print).
3. Pearson TS, Akman C, Hinton VJ, Engelstad K, De Vivo DC. Phenotypic spectrum of glucose transporter type 1 deficiency syndrome (Glut1 DS). *Curr Neurol Neurosci Rep*. 2013;13:342-350.
4. Leen WG, Klepper J, Verbeek MM, et al. Glucose transporter-1 deficiency syndrome: the expanding clinical and genetic spectrum of a treatable disorder. *Brain*. 2010;133:655-670.
5. Joshi C, Greenberg CR, De Vivo D, Dong W, Chan-Lui W, Booth FA. GLUT1 deficiency without epilepsy: yet another case. *J Child Neurol*. 2008;23:832-834.
6. Kotagal V. Acetazolamide-responsive ataxia. *Semin Neurol*. 2012;32:533-537.
7. Pons R, Collins A, Rotstein M, Engelstad K, De Vivo DC. The spectrum of movement disorders in Glut-1 deficiency. *Mov Disord*. 2010;25:275-281.
8. Anheim M, Maillart E, Vuillaumier-Barrot S, et al. Excellent response to acetazolamide in a case of paroxysmal dyskinesias due to GLUT1-deficiency. *J Neurol*. 2011;258:316-317.
9. Brockmann K. The expanding phenotype of GLUT1-deficiency syndrome. *Brain Dev*. 2009;31:545-552.
10. Ito Y, Oguni H, Ito S, Oguni M, Osawa M. A modified Atkins diet is promising as a treatment for glucose transporter type 1 deficiency syndrome. *Dev Med Child Neurol*. 2011;53:658-663.

Expanding the phenotypic spectrum of *TUBB4A*-associated hypomyelinating leukoencephalopathies

Satoko Miyatake, MD, PhD
Hitoshi Osaka, MD, PhD
Masaaki Shiina, MD, PhD
Masayuki Sasaki, MD, PhD
Jun-ichi Takanashi, MD, PhD
Kazuhiro Haginoya, MD, PhD
Takahito Wada, MD, PhD
Masafumi Morimoto, MD, PhD
Naoki Ando, MD, PhD
Yoji Ikuta, MD
Mitsuko Nakashima, MD, PhD
Yoshinori Tsurusaki, PhD
Noriko Miyake, MD, PhD
Kazuhiro Ogata, MD, PhD
Naomichi Matsumoto, MD, PhD
Hirotomo Saitsu, MD, PhD

Correspondence to Dr. Matsumoto: naomat@yokohama-cu.ac.jp or Dr. Saitsu: hsaitsu@yokohama-cu.ac.jp

Supplemental data at Neurology.org

ABSTRACT

Objective: We performed whole-exome sequencing analysis of patients with genetically unsolved hypomyelinating leukoencephalopathies, identifying 8 patients with *TUBB4A* mutations and allowing the phenotypic spectrum of *TUBB4A* mutations to be investigated.

Methods: Fourteen patients with hypomyelinating leukoencephalopathies, 7 clinically diagnosed with hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC), and 7 with unclassified hypomyelinating leukoencephalopathy, were analyzed by whole-exome sequencing. The effect of the mutations on microtubule assembly was examined by mapping altered amino acids onto 3-dimensional models of the αβ-tubulin heterodimer.

Results: Six heterozygous missense mutations in *TUBB4A*, 5 of which are novel, were identified in 8 patients (6/7 patients with H-ABC [the remaining patient is an atypical case] and 2/7 patients with unclassified hypomyelinating leukoencephalopathy). In 4 cases with parental samples available, the mutations occurred de novo. Analysis of 3-dimensional models revealed that the p.Glu410Lys mutation, identified in patients with unclassified hypomyelinating leukoencephalopathy, directly impairs motor protein and/or microtubule-associated protein interactions with microtubules, whereas the other mutations affect longitudinal interactions for maintaining αβ-tubulin structure, suggesting different mechanisms in tubulin function impairment. In patients with the p.Glu410Lys mutation, basal ganglia atrophy was unobserved or minimal although extrapyramidal features were detected, suggesting its functional impairment.

Conclusions: *TUBB4A* mutations cause typical H-ABC. Furthermore, *TUBB4A* mutations associate cases of unclassified hypomyelinating leukoencephalopathies with morphologically retained but functionally impaired basal ganglia, suggesting that *TUBB4A*-related hypomyelinating leukoencephalopathies encompass a broader clinical spectrum than previously expected. Extrapyramidal findings may be a key for consideration of *TUBB4A* mutations in hypomyelinating leukoencephalopathies. ***Neurology*® 2014;82:2230–2237**

GLOSSARY

4H = hypomyelination, hypodontia, and hypogonadotropic hypogonadism; **H-ABC** = hypomyelination with atrophy of the basal ganglia and cerebellum; **MAP** = microtubule-associated protein; **MREI** = Met-Arg-Glu-Ile; ***TUBB4A*** = tubulin, beta 4A class IVa.

Leukoencephalopathies are a heterogeneous group of disorders affecting the white matter of the brain. It is estimated that approximately 30% to 40% of patients with leukoencephalopathy remain without a specific diagnosis despite extensive investigations.[1] Brain MRI aids diagnosis because distinct MRI patterns enable easier detection of white matter abnormalities and successful categorization.[1,2] Moreover, recent advances in whole-exome sequencing have improved understanding of these clinically defined/undefined disease entities by identifying genetic causes and their phenotypic spectrum. For example, the majority of cases with hypomyelination,

From the Departments of Human Genetics (S.M., M.N., Y.T., N. Miyake, N. Matsumoto, H.S.) and Biochemistry (M. Shiina, K.O.), Yokohama City University Graduate School of Medicine; Division of Neurology (H.O.), Clinical Research Institute, Kanagawa Children's Medical Center, Yokohama; Department of Pediatrics (H.O.), Jichi Medical School, Tochigi; Department of Child Neurology (M. Sasaki), National Center of Neurology and Psychiatry, Tokyo; Department of Pediatrics (J.-i.T.), Kameda Medical Center, Chiba; Department of Pediatric Neurology (K.H.), Takuto Rehabilitation Center for Children, Sendai; Genetic Counselling and Clinical Research Unit (T.W.), Kyoto University School of Public Health; Department of Pediatrics (M.M.), Graduate School of Medical Science, Kyoto Prefectural University of Medicine; Department of Neonatology and Pediatrics (N.A.), Nagoya City University Graduate School of Medical Sciences; and Department of Neurology (Y.I.), Tokyo Metropolitan Children's Medical Center, Japan.

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.

hypodontia, and hypogonadotropic hypogonadism (4H syndrome),[3–5] tremor-ataxia with central hypomyelination leukodystrophy (TACH),[6] leukodystrophy with oligodontia (LO),[7,8] or hypomyelination with cerebellar atrophy and hypoplasia of the corpus callosum (HCAHC),[9] which was described in Japan, share some clinical overlap and have *POLR3A* or *POLR3B* mutations in common.[10–14]

Hypomyelination with atrophy of the basal ganglia and cerebellum (H-ABC)[15,16] is characterized by early-onset motor regression and/or delay followed by extrapyramidal symptoms, distinguishing H-ABC from other hypomyelinating leukoencephalopathies caused by *POLR3A* or *POLR3B* mutations. A recurrent de novo *TUBB4A* mutation was recently reported in 11 patients with H-ABC.[17] Of note, *TUBB4A* mutations also cause autosomal dominant DYT4 dystonia,[18,19] a condition that presents with normal brain MRI findings. This suggests that in addition to H-ABC, *TUBB4A* mutations may be widely related to other hypomyelinating leukoencephalopathies. Herein, we describe 8 patients with *TUBB4A* mutations identified by whole-exome sequencing, clarifying their phenotypic spectrum.

METHODS

Study subjects. Fourteen patients with molecularly undiagnosed hypomyelinating leukoencephalopathy were included in the study. Patients were diagnosed based on clinical symptoms and brain MRI findings. Among the 14 patients, 7 were clinically diagnosed with H-ABC and 7 with hypomyelinating leukoencephalopathy that did not meet the criteria for H-ABC, 4H syndrome, or Pelizaeus-Merzbacher disease. Patients with *POLR3A* or *POLR3B* mutations were excluded from this cohort. When available, parental samples were also tested in mutation-positive patients.

Standard protocol approvals, registrations, and patient consents. Experimental protocols were approved by the Committee for Ethical Issues at Yokohama City University School of Medicine. Written informed consent was obtained from all patients or their parents.

Mutation analysis. We performed whole-exome sequencing in 14 patients. Genomic DNA was captured using the SureSelect^{XT} Human All Exon 50 Mb (v3) or 51 Mb (v4) Kit (Agilent Technologies, Santa Clara, CA) and sequenced on either the GAIIx platform (Illumina, San Diego, CA) with 108–base pair paired-end reads or HiSeq2000 (Illumina) with 101–base pair paired-end reads. After filtering against dbSNP135 and 91 in-house normal control exomes, rare protein-altering and splice-site variant calls were obtained for each patient. We identified *TUBB4A* mutation calls and confirmed these mutations by Sanger sequencing. In 4 of 8 patients with *TUBB4A* mutations, parental samples were analyzed by Sanger sequencing to determine the mode of inheritance.

Three-dimensional structure modeling. To determine the effect of *TUBB4A* mutations on microtubule assembly, we mapped mutation positions onto the 3-dimensional structure of the αβ-tubulin heterodimer (Protein Data Bank code 1JFF)[20] and examined their interaction with surrounding molecules.

RESULTS

Identification of *TUBB4A* mutations. Whole-exome sequencing identified 6 heterozygous missense mutations in *TUBB4A*, in 6 of 7 patients with H-ABC (85.7%) and 2 of 7 patients with unclassified hypomyelinating leukoencephalopathy (28.6%) (see table 1 and tables e-1 and e-2 on the *Neurology*® Web site at Neurology.org). Two mutations, c.1228G>A (p.Glu410Lys) and c.745G>A (p.Asp249Asn), were identified in 2 unrelated patients. Two hypomyelinating patients with similar clinical features as those previously reported,[9] carried the c.1228G>A mutation. The c.745G>A mutation was a recurrent mutation reported in patients with H-ABC.[17] The other 5 mutations were novel. None of the mutations were registered in the National Heart, Lung, and Blood Institute Exome Sequencing Project (ESP6500), 1000 Genomes, or our 575 in-house control exomes. The c.5G>A (p.Arg2Gln) missense mutation, identified in a patient with H-ABC, alters Arg2 to Gln. Arg2 is located within the highly conserved, amino-terminal β-tubulin tetrapeptide Met-Arg-Glu-Ile (MREI) motif and is involved in autoregulatory mechanisms for β-tubulin stability. Notably, Arg2 is altered to Gly in a large family with DYT4.[18,19] All of the mutations occur within highly conserved residues, from yeast to human, and among human β-tubulins (figure 1). GERP (Genomic Evolutionary Rate Profiling) scores were high for all mutated residues, and Web-based prediction programs identified all mutations as pathogenic (table e-1). In 4 patients with parental samples available, the mutations occurred de novo (table e-1). In 2 patients, only the mother's sample was available and confirmed as mutation-negative.

Three-dimensional structural modeling analysis. Tubulin heterodimers polymerize longitudinally in a head-to-tail manner, forming protofilaments, which then laterally interact with each other to form microtubules (figure 2). Some mutations fall within longitudinal interaction interfaces, whereas others are near interaction regions for motor proteins and microtubule-associated proteins (MAPs).[21,22] Thr178 of β-tubulin is located at a longitudinal interheterodimer interface, in proximity to the guanine nucleotide–binding pocket of β-tubulin (figure 2). This residue is reportedly important for regulation of αβ-tubulin heterodimer polymerization with GTP[23,24]; therefore, the Thr178Arg mutation may affect the polymerization process. Arg2 and Asp249 of β-tubulin are

Table 1 **Clinical features of the patients**

	Patient 1[9]	Patient 2[9]	Patient 3	Patient 4[26]	Patient 5[27]	Patient 6	Patient 7	Patient 8
Current age, y, sex	23, M	41, M	15, F	12, M	16, M	10, M	4, M	1, F
Mutation	c.1228G>A	c.1228G>A	c.5G>A	c.745G>A	c.1162A>G	c.745G>A	c.533C>G	c.785G>A
Protein alteration	p.Glu410Lys	p.Glu410Lys	p.Arg2Gln	p.Asp249Asn	p.Met388Val	p.Asp249Asn	p.Thr178Arg	p.Arg262His
Initial diagnosis	Unclassified hypomyelinating leukoencephalopathy[a]	Unclassified hypomyelinating leukoencephalopathy[a]	H-ABC	H-ABC	H-ABC	H-ABC	H-ABC	H-ABC
Age at onset, mo	12	12	1.5	18	3	19	6	2
Maximum motor milestone	Unsupported unstable walking	Unsupported unstable walking	No head control	Walking for a few steps	Rolling over	Supported walking	No head control	No head control
Onset of motor deterioration	10 y	20 y	ND	18 mo	3 mo	19 mo	ND	ND
Intellectual disability	Mild	Moderate	Severe	Severe	Severe	Severe	Severe	Moderate
Motor signs								
Ataxia	+	+	ND	+	ND	+	ND	ND
Tremor	+	+	−	−	−	+	−	ND
Spasticity	+	+	+	+	+	+	ND	+
Babinski sign	+	+	−	+	ND	+	−	+
Rigidity	+	+	+	+	+	+	+	−
Choreoathetosis	−	−	+	+	+	−	−	−
Dystonia	+	+	+	+	+	+	−	−
Brain MRI findings								
Hypomyelination	+	+	+	+	+	+	+	+
Atrophy of the basal ganglia	−	±	+	+	+	+	+	+
Atrophy of the cerebellum	+	+	+	+	+	+	+	−
Atrophy of the corpus callosum	+	+	+	+	+	+	+	−

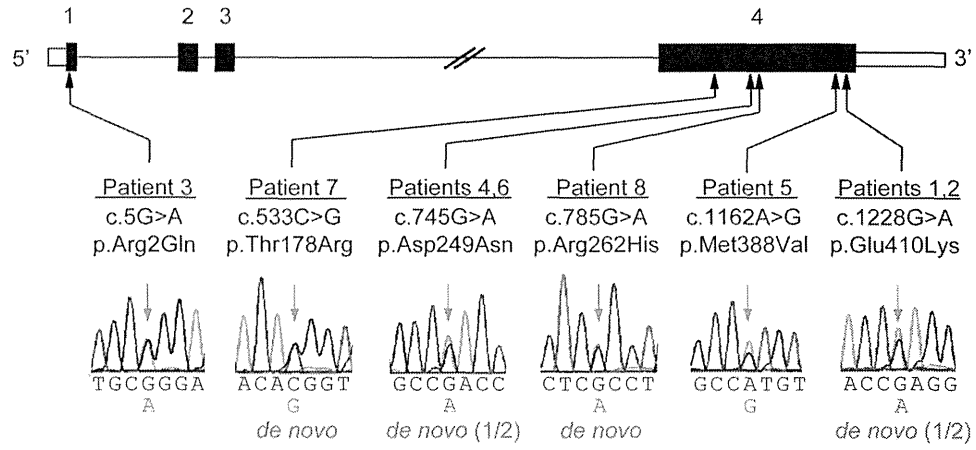
Abbreviations: H-ABC = hypomyelination with atrophy of the basal ganglia and cerebellum; ND = not determined.
Symbols: + = present; − = absent; ± = minimally detected.
[a] Unclassified hypomyelinating leukoencephalopathy: did not meet the criteria for H-ABC, 4H syndrome (hypomyelination, hypodontia, and hypogonadotropic hypogonadism), or Pelizaeus-Merzbacher disease.

located at an intraheterodimer interface (figures 2 and e-1A). These residues stabilize the β-tubulin T7 loop region, which interacts with α-tubulin within a heterodimer (figure e-1A), indicating that the p.Arg2Gln and p.Asp249Asn mutations may affect tubulin heterodimerization. Glu410 is located on the exposed outer surface that mediates interactions with motor proteins and/or MAPs (figures 2 and e-1B).[21,22] This residue is crucial for the kinesin-microtubule interaction, and thus the p.Glu410Lys mutation may directly impair motor protein and/or MAP interactions with microtubules. Arg262 and Met388 are located near the intra- and interheterodimer interfaces, respectively, and both are also near the interaction region for motor proteins and/or MAPs (figures 2 and e-1, B and C). Arg262 is involved in the hydrophobic core with residues from a loop that interacts with the α-tubulin subunit within the heterodimer, and from helix H12, which interacts with motor proteins and/or MAPs (figures 2 and e-1B). Met388 is involved in the hydrophobic core with residues from helix H11, which interacts with the α-tubulin subunit in the neighboring heterodimer, and from helix H12 (figures 2 and e-1C).[25] Thus, the p.Arg262His and the p.Met388Val mutations may destabilize the hydrophobic core and potentially affect the tertiary structure, resulting in impairment of longitudinal intra- and interheterodimer tubulin interactions, respectively, and/or interaction with motor proteins and/or MAPs.

Clinical features. Clinical information on patients with *TUBB4A* mutations is presented in tables 1 and e-2, and brain MRIs are shown in figures 3 and e-2.

The mean age at onset was 9.2 months, although the age at onset was varied. Initial motor development also varied, with some acquiring unsupported but

Figure 1 *TUBB4A* mutations in patients with hypomyelinating leukoencephalopathy



Amino acid change	Q	R	N	H	V	K
H. sapiens_TUBB4A	MREIVHL	VSDTVVE	LNADLRK	PFPRLHF	FTAMFRR	EFTEAES
M. musculus_TUBB4A	MREIVHL	VSDTVVE	LNADLRK	PFPRLHF	FTAMFRR	EFTEAES
B. Taurus_TUBB4A	MREIVHL	VSDTVVE	LNADLRK	PFPRLHF	FTAMFRR	EFTEAES
X. tropicalis_TUBB4A	MREIVHL	VSDTVVE	LNADLRK	PFPRLHF	FTAMFRR	EFTEAES
D. rerio_TUBB2C	MREIVHL	VSDTVVE	LNADLRK	PFPRLHF	FTAMFRR	EFTEAES
TUBB4B_Human	MREIVHL	VSDTVVE	LNADLRK	PFPRLHF	FTAMFRR	EFTEAES
TUBB2B_Human	MREIVHI	VSDTVVE	LNADLRK	PFPRLHF	FTAMFRR	EFTEAES
TBB1_Human	MREIVHI	VSDTVVE	LNADLRK	PFPRLHF	FSAMFRR	EFGEAES
TBB_YEAST	MREIVHI	TSDTVVE	LNADLRK	PFPRLHF	FSAMFRR	EFSEAES

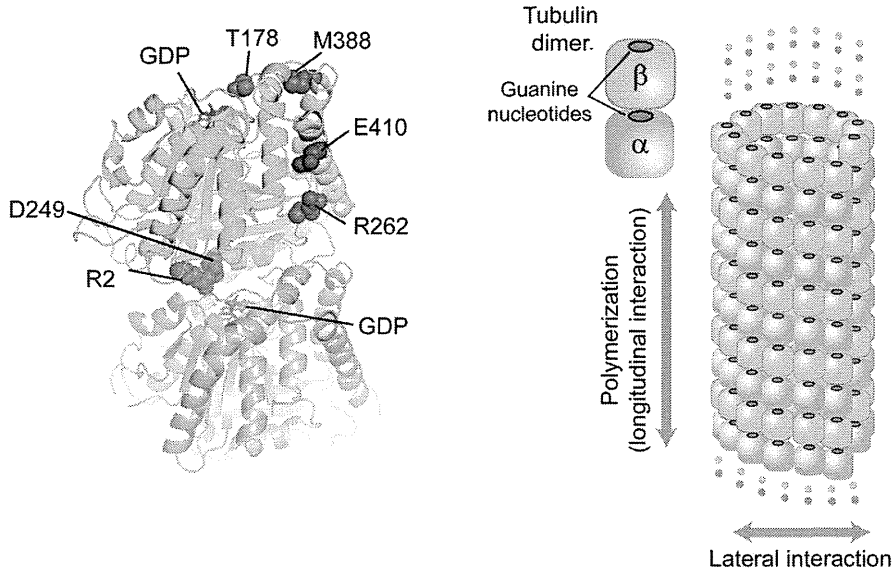
TUBB4A schematic with the 6 mutations is presented. Untranslated regions and coding regions are shown in white and black rectangles, respectively. All mutations occur at evolutionarily conserved amino acids. Homologous sequences were aligned using CLUSTALW (http://www.genome.jp/tools/clustalw/).

unsteady walking and others never acquiring head control. The maximum motor milestone of these patients was unstable short walking. The clinical course appeared milder in patients with an older age at onset. This tendency was most prominent in patients initially diagnosed with unclassified hypomyelinating leukoencephalopathy. For example, the onset of motor deterioration started in the first or second decades in these patients but was between 0 and 3 years old in patients with typical H-ABC. Intellectual disability was mild to moderate in the former but mostly severe in the latter patients.

All clinically evaluated patients with *TUBB4A* mutations demonstrated cerebellar ataxia and spasticity. Except for patient 8, all demonstrated extrapyramidal features such as rigidity, dystonia, or choreoathetosis. In patient 1, dystonia was prominent compared with other hypomyelination patients with either *POLR3A* or *POLR3B* mutations.[9,11] Patient 8 was 1 year old at the time of the study, and brain MRI showed a relatively small but still well-retained putamen compared with healthy subjects of the same age, suggesting that extrapyramidal features may not yet have developed but would likely express as the basal ganglia atrophy progressed. Notably, both hypomyelinating patients with either very mild basal ganglia atrophy (patient 2) or none identifiable (patient 1) demonstrated extrapyramidal signs, suggesting that the basal ganglia may be impaired functionally in these patients as well as other patients with typical H-ABC. Case reports are available in appendix e-1. Patients 1 and 2,[9] 4,[26] and 5[27] were previously described. Retrospectively, patient 2 might be diagnosed with atypical H-ABC because minimal basal ganglia atrophy cannot be excluded. In the patient with H-ABC with no *TUBB4A* mutation, the atrophy of basal ganglia was very mild compared with that of patients with typical H-ABC. However, clinical symptoms are very severe with neither head control nor sitting at 12 years, suggesting that the patient has atypical H-ABC.

DISCUSSION The β- and α-tubulins are major components of microtubules. Microtubules have essential roles in many cellular processes including mitosis, intracellular transport, asymmetric neuronal morphology, and ciliary and flagellar motility.[28] Multiple β-tubulin isotypes are present, with high homology (differing primarily at 15–20 amino acids within the C terminus), and expressed differentially in a tissue-dependent manner.[29] Certain isotypes, namely, β-tubulin isotypes 2A, 2B, 3, and 4A, are neuron-specific proteins and highly expressed in brain.[28] In the nervous system, microtubules provide structure, generate force necessary for neuronal migration, and serve as scaffolds for motor proteins and/or MAPs to transport cargo.[30] In addition to *TUBB4A*-associated leukoencephalopathies[17] and dystonia,[18,19] *TUBA1A*, *TUBB2B*, and *TUBB3*

Figure 2 **Structural prediction of *TUBB4A* mutations in the αβ-tubulin heterodimer**



Mapping of disease-causing amino acid mutations on the αβ-tubulin heterodimer (Protein Data Bank code 1JFF) crystal structure, with schematic representation of a tubulin dimer (left) and microtubule segment (right). The α- and β-tubulins are colored gray and green, respectively. Left: The longitudinal interheterodimer interface of β-tubulin (which interacts with α-tubulin in a neighboring αβ heterodimer) is colored pink,[24] and the β-tubulin microtubule-associated protein and motor protein interaction region is colored cyan.[21,22] Side chains of residues altered by the mutations are shown in space-filling representation in red. Helices, β-sheets, and loops are shown as ribbons, arrows, and threads, respectively, and nucleotides are blue sticks. Right: Tubulin heterodimers polymerize longitudinally to form protofilaments (longitudinal interaction), then laterally interact with each other to form microtubules (lateral interaction). Blue circles represent guanine nucleotide–binding pockets of α- and β-tubulins.

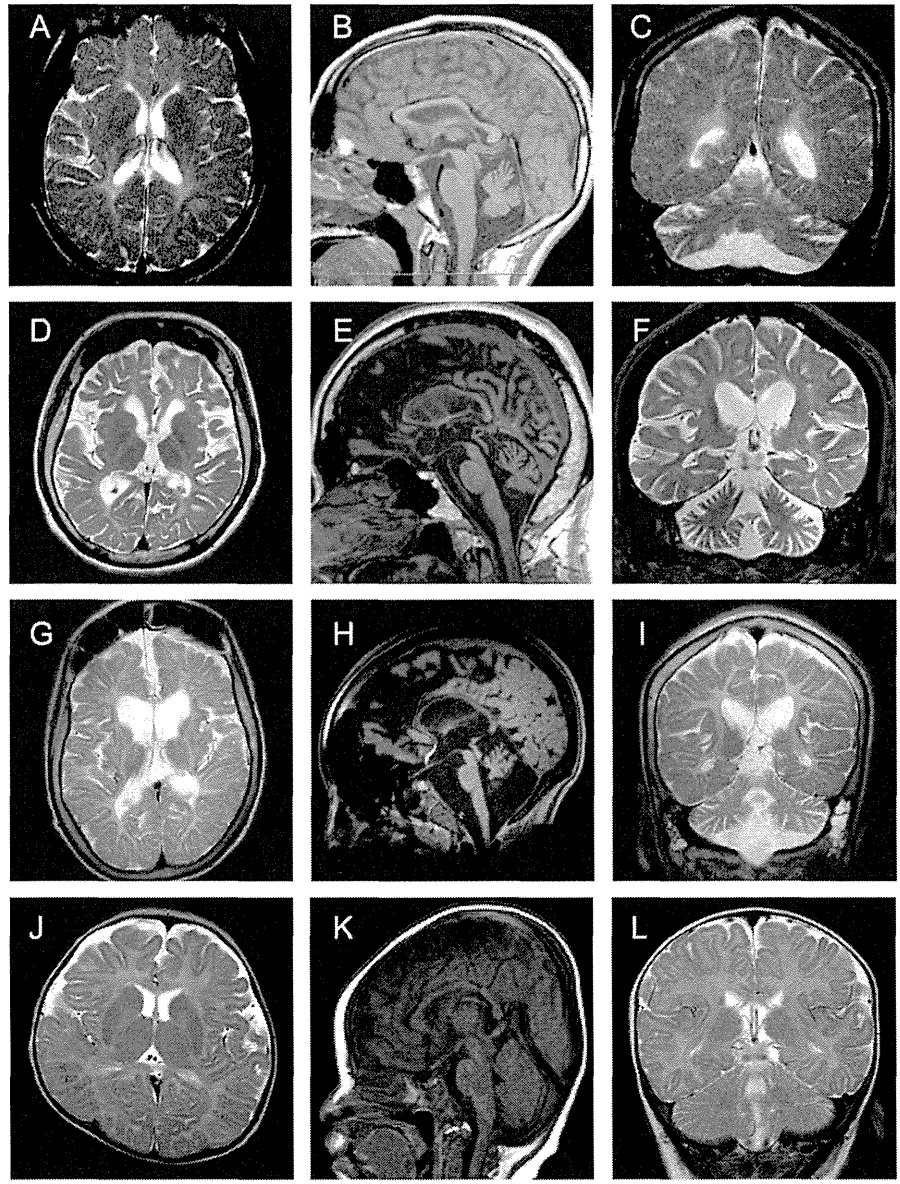
mutations are reported to cause the spectrum of neurologic disorders resulting from neural migration, differentiation, and axon guidance and maintenance abnormalities,[25] demonstrating the importance of αβ-tubulin heterodimers in the nervous system.

In this study, we identified 6 missense *TUBB4A* mutations, 5 of which are novel, in 6 of 7 patients with H-ABC and 2 of 7 patients initially diagnosed with unclassified hypomyelinating leukoencephalopathy. Of the patients with H-ABC, all 6 patients with *TUBB4A* mutations showed typical H-ABC, supporting that H-ABC is a distinct disease entity caused by *TUBB4A* abnormality. We did not detect any *TUBB4A* mutations in one patient with atypical H-ABC. This may be because this patient has a clinically similar, but different disease, possibly caused by a different mutated gene.

We report a *TUBB4A* mutation in 2 patients with preserved basal ganglia. Their brain MRI findings are similar to patients with *POLR3A* or *POLR3B* mutations, rather than H-ABC. However, it is notable that both patients showed apparent extrapyramidal signs, to suggest functional impairment. Accompanying extrapyramidal features are extremely atypical in patients with either *POLR3A* or *POLR3B* mutations.[9,11] Furthermore, comparing these 2 patients with other typical H-ABC patients with *TUBB4A* mutations, patients with minimal basal ganglia atrophy tend to have a milder clinical course. Both patients have a recurrent missense mutation, c.1228G>A (p.Glu410Lys). Based on our 3-dimensional modeling analysis, the Glu410Lys mutation is predicted to directly impair motor protein and/or MAP interactions with microtubules, while the other mutations identified in patients with typical H-ABC may affect longitudinal interactions for maintaining αβ-tubulin heterodimerization/polymerization. Different effects of the *TUBB4A* mutations on tubulin function may lead to this phenotypic variation. Supporting this hypothesis, the p.Glu410Lys mutation in *TUBB3*, which also directly alters a kinesin motor protein binding site in β-tubulin isotype 3, demonstrates clinically distinct features compared with the other mutations.[30] Therefore, the p.Glu410Lys mutation in *TUBB4A* may contribute to the milder end of the phenotypic spectrum of *TUBB4A* mutations. Additional patients with *TUBB4A* mutations are needed to clinically confirm mutational consequences.

Another important finding is that one of the patients with H-ABC had a p.Arg2Gln mutation, since the p.Arg2Gly mutation has recently been identified in patients from a large DYT4 family.[18,19] DYT4 was described in 1985 in an Australian family that had emigrated from England as whispering dysphonia and generalized dystonia. To date, no other pedigrees

Figure 3 **Brain MRI of patients with *TUBB4A* mutations**



Axial T2-weighted (A, D, G, J), sagittal T1-weighted (B, E, H, K), and coronal T2-weighted (C, F, I, L) images. Patient 1 at 14 years of age (A); patient 1 at 16 years (B, C); patient 2 at 38 years (D-F); patient 3 at 13 years (G-I); and patient 8 at 7 months of age (J-L). All patients show diffuse cerebral white matter hypomyelination with normal (J), mildly reduced (A), or considerably reduced (D, G) white matter volumes. In patient 1, cerebral white matter hypomyelination is unchanged, comparing at 14 (A) and 16 (B, C) years of age. In patient 1, the putamen and the head of the caudate nucleus are normal in size (A). In patient 2, minimal putamen atrophy cannot be excluded (D). The putamen and the head of the caudate nucleus are small or hardly recognizable in patient 3 (G). In patient 8, the putamen is slightly small compared with a healthy control at the same age (J). The globus pallidus and thalamus are normal in size (A, D, G, J). Atrophy of the cerebellar vermis and hemisphere, and corpus callosum was variably observed in 4 patients, but not patient 8 (B, C, E, F, H, I, K, L).

with this phenotype have been reported worldwide.[18] The symptoms typically emerge in the third decade, following a highly penetrant, autosomal dominant mode of inheritance.[31] Brain MRI demonstrates normal structural findings. Arg2 resides within the autoregulatory MREI domain of β-tubulin 4A, which is necessary for autoregulation of the β-tubulin messenger RNA transcript. Site-directed mutagenesis shows that any Arg2 substitution leads to loss of autoregulated instability and increased mutant tubulin subunit levels.[32] Thus, mutations in the MREI domain have been assumed to cause DYT4 rather than H-ABC, because of the different impact on *TUBB4A*.[17] However, our study shows that mutations in the MREI domain can also cause the H-ABC phenotype. The phenotypic difference between the p.Arg2Gly and p.Arg2Gln mutations remains unsolved. Because DYT4 is an extremely rare

syndrome that has only been described in one large pedigree so far, patients of the family may have another modifying factor(s) to spare cerebral white matter abnormalities.

Diffuse hypomyelination syndromes are a heterogeneous group of disorders with overlapping clinical features. Currently, they are categorized based on brain MRI findings, which is very useful in clinical practice. Basal ganglia atrophy specifically distinguishes H-ABC from other hypomyelination disorders. Our study shows that *TUBB4A* mutations associate not only with the typical H-ABC cases but also with some hypomyelinating patients with retained basal ganglia, although notably all patients with *TUBB4A* mutations have extrapyramidal features in common. Our study implies that *TUBB4A* may cause hypomyelinating leukoencephalopathies with either a morphologically or a functionally impaired basal ganglia. Extrapyramidal features may be a key for clinicians to examine *TUBB4A* mutations in genetically unsolved hypomyelinating leukoencephalopathies.

AUTHOR CONTRIBUTIONS

Satoko Miyatake: genetic and clinical data analysis, data interpretation, and drafting/revising of the manuscript. Hitoshi Osaka: clinical data analysis and sample collection. Masaaki Shiina: structural data analysis. Masayuki Sasaki, Jun-ichi Takanashi, Kazuhiro Haginoya, Takahito Wada, Masafumi Morimoto, Naoki Ando, and Yoji Ikuta: clinical data analysis and sample collection. Mitsuko Nakashima, Yoshinori Tsurusaki, and Noriko Miyake: genetic data analysis. Kazuhiro Ogata: structural data analysis. Naomichi Matsumoto: study concept and design, data interpretation, and drafting/revising of the manuscript. Hirotomo Saitsu: study concept and design, genetic data analysis, data interpretation, and drafting/revising of the manuscript.

ACKNOWLEDGMENT

The authors thank all of the participants for their cooperation in this research, and Dr. K. Nishiyama, Ms. K. Takabe, Mr. T. Miyama, Ms. A. Narita, Ms. N. Watanabe, and Ms. S. Sugimoto, from the Department of Human Genetics, Yokohama City University Graduate School of Medicine, for their technical assistance.

STUDY FUNDING

Supported by the Ministry of Health, Labour and Welfare of Japan; the Japan Society for the Promotion of Science (a Grant-in-Aid for Scientific Research [B] [25293085, 25293235]; and a Grant-in-Aid for Scientific Research [A] [13313587]); the Takeda Science Foundation; the fund for Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program in the Project for Developing Innovation Systems; the Strategic Research Program for Brain Sciences (11105137); and a Grant-in-Aid for Scientific Research on Innovative Areas (Transcription Cycle) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (12024421).

DISCLOSURE

S. Miyatake is funded by research grants from the Yokohama Foundation for Advancement of Medical Science. H. Osaka is funded by research grants from the Ministry of Health, Labour and Welfare of Japan (Research on Rare and Intractable Diseases [H24-Nanchitou-Ippan-072]). M. Shiina and M. Sasaki report no disclosures relevant to the manuscript. J. Takanashi is funded by research grants from the Ministry of Health, Labour and Welfare of Japan (Research on Rare and Intractable Diseases [H24-Nanchitou-Ippan-072]). K. Haginoya, T. Wada, M. Morimoto, N. Ando, Y. Ikuta, M. Nakashima, and Y. Tsurusaki report no disclosures relevant to the manuscript. N. Miyake is funded by research grants from the Ministry of Health, Labour and Welfare of Japan, a Grant-in-Aid for Scientific Research (B) from the Japan Society for the Promotion of Science, and a research grant from the Takeda Science Foundation. K. Ogata is supported by a Grant-in-Aid for Scientific Research on Innovative Areas (Transcription Cycle) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. N. Matsumoto is supported by grants from the Ministry of Health, Labour and Welfare of Japan, a Grant-in-Aid for Scientific Research (A) from the Japan Society for the Promotion of Science, the Takeda Science Foundation, the fund for Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program in the Project for Developing Innovation Systems, the Strategic Research Program for Brain Sciences, and a Grant-in-Aid for Scientific Research on Innovative Areas (Transcription Cycle) from the Ministry of Education, Culture, Sports, Science and Technology of Japan. H. Saitsu is funded by research grants from a Grant-in-Aid for Scientific Research (B) from the Japan Society for the Promotion of Science, and a research grant from the Takeda Science Foundation. Go to Neurology.org for full disclosures.

Received October 10, 2013. Accepted in final form March 20, 2014.

REFERENCES

1. Schiffmann R, van der Knaap MS. Invited article: an MRI-based approach to the diagnosis of white matter disorders. Neurology 2009;72:750–759.
2. Steenweg ME, Vanderver A, Blaser S, et al. Magnetic resonance imaging pattern recognition in hypomyelinating disorders. Brain 2010;133:2971–2982.
3. Wolf NI, Harting I, Boltshauser E, et al. Leukoencephalopathy with ataxia, hypodontia, and hypomyelination. Neurology 2005;64:1461–1464.
4. Timmons M, Tsokos M, Asab MA, et al. Peripheral and central hypomyelination with hypogonadotropic hypogonadism and hypodontia. Neurology 2006;67:2066–2069.
5. Vazquez-Lopez M, Ruiz-Martin Y, de Castro-Castro P, Garzo-Fernandez C, Martin-del Valle F, Marquez-de la Plata L. Central hypomyelination, hypogonadotrophic hypogonadism and hypodontia: a new leukodystrophy [in Spanish]. Rev Neurol 2008;47:204–208.
6. Bernard G, Thiffault I, Tetreault M, et al. Tremor-ataxia with central hypomyelination (TACH) leukodystrophy maps to chromosome 10q22.3-10q23.31. Neurogenetics 2010;11:457–464.
7. Atrouni S, Daraze A, Tamraz J, Cassia A, Caillaud C, Megarbane A. Leukodystrophy associated with oligodontia in a large inbred family: fortuitous association or new entity? Am J Med Genet A 2003;118A:76–81.
8. Chouery E, Delague V, Jalkh N, et al. A whole-genome scan in a large family with leukodystrophy and oligodontia reveals linkage to 10q22. Neurogenetics 2011;12:73–78.
9. Sasaki M, Takanashi J, Tada H, Sakuma H, Furushima W, Sato N. Diffuse cerebral hypomyelination with cerebellar atrophy and hypoplasia of the corpus callosum. Brain Dev 2009;31:582–587.
10. Bernard G, Chouery E, Putorti ML, et al. Mutations of POLR3A encoding a catalytic subunit of RNA polymerase Pol III cause a recessive hypomyelinating leukodystrophy. Am J Hum Genet 2011;89:415–423.
11. Saitsu H, Osaka H, Sasaki M, et al. Mutations in POLR3A and POLR3B encoding RNA polymerase III subunits cause an autosomal-recessive hypomyelinating leukoencephalopathy. Am J Hum Genet 2011;89:644–651.
12. Tetreault M, Choquet K, Orcesi S, et al. Recessive mutations in POLR3B, encoding the second largest subunit of Pol III, cause a rare hypomyelinating leukodystrophy. Am J Hum Genet 2011;89:652–655.

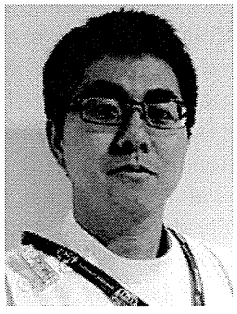
13. Potic A, Brais B, Choquet K, Schiffmann R, Bernard G. 4H syndrome with late-onset growth hormone deficiency caused by POLR3A mutations. Arch Neurol 2012;69: 920–923.
14. Daoud H, Tetreault M, Gibson W, et al. Mutations in POLR3A and POLR3B are a major cause of hypomyelinating leukodystrophies with or without dental abnormalities and/or hypogonadotropic hypogonadism. J Med Genet 2013;50:194–197.
15. van der Knaap MS, Naidu S, Pouwels PJ, et al. New syndrome characterized by hypomyelination with atrophy of the basal ganglia and cerebellum. AJNR Am J Neuroradiol 2002;23:1466–1474.
16. van der Knaap MS, Linnankivi T, Paetau A, et al. Hypomyelination with atrophy of the basal ganglia and cerebellum: follow-up and pathology. Neurology 2007;69: 166–171.
17. Simons C, Wolf NI, McNeil N, et al. A de novo mutation in the beta-tubulin gene TUBB4A results in the leukoencephalopathy hypomyelination with atrophy of the basal ganglia and cerebellum. Am J Hum Genet 2013;92: 767–773.
18. Hersheson J, Mencacci NE, Davis M, et al. Mutations in the autoregulatory domain of beta-tubulin 4a cause hereditary dystonia. Ann Neurol 2013;73:546–553.
19. Lohmann K, Wilcox RA, Winkler S, et al. Whispering dysphonia (DYT4 dystonia) is caused by a mutation in the TUBB4 gene. Ann Neurol 2013;73:537–545.
20. Lowe J, Li H, Downing KH, Nogales E. Refined structure of alpha beta-tubulin at 3.5 Å resolution. J Mol Biol 2001; 313:1045–1057.
21. Uchimura S, Oguchi Y, Katsuki M, et al. Identification of a strong binding site for kinesin on the microtubule using mutant analysis of tubulin. EMBO J 2006;25:5932–5941.
22. Al-Bassam J, Ozer RS, Safer D, Halpain S, Milligan RA. MAP2 and tau bind longitudinally along the outer ridges of microtubule protofilaments. J Cell Biol 2002;157: 1187–1196.
23. Nawrotek A, Knossow M, Gigant B. The determinants that govern microtubule assembly from the atomic structure of GTP-tubulin. J Mol Biol 2011;412:35–42.
24. Nogales E, Whittaker M, Milligan RA, Downing KH. High-resolution model of the microtubule. Cell 1999; 96:79–88.
25. Tischfield MA, Cederquist GY, Gupta ML Jr, Engle EC. Phenotypic spectrum of the tubulin-related disorders and functional implications of disease-causing mutations. Curr Opin Genet Dev 2011;21:286–294.
26. Wakusawa K, Haginoya K, Kitamura T, et al. Effective treatment with levodopa and carbidopa for hypomyelination with atrophy of the basal ganglia and cerebellum. Tohoku J Exp Med 2006;209:163–167.
27. Hattori A, Ando N, Fujimoto S, Kobayashi S, Ishikawa T, Togari H. A boy with hypomyelination with atrophy of the basal ganglia and cerebellum [in Japanese]. No To Hattatsu 2010;42:42–44.
28. Leandro-Garcia LJ, Leskela S, Landa I, et al. Tumoral and tissue-specific expression of the major human beta-tubulin isotypes. Cytoskeleton 2010;67:214–223.
29. Sullivan KF, Cleveland DW. Sequence of a highly divergent beta tubulin gene reveals regional heterogeneity in the beta tubulin polypeptide. J Cell Biol 1984;99:1754–1760.
30. Chew S, Balasubramanian R, Chan WM, et al. A novel syndrome caused by the E410K amino acid substitution in the neuronal beta-tubulin isotype 3. Brain 2013;136: 522–535.
31. Parker N. Hereditary whispering dysphonia. J Neurol Neurosurg Psychiatry 1985;48:218–224.
32. Yen TJ, Machlin PS, Cleveland DW. Autoregulated instability of beta-tubulin mRNAs by recognition of the nascent amino terminus of beta-tubulin. Nature 1988;334: 580–585.

BRIEF COMMUNICATION

PIGO mutations in intractable epilepsy and severe developmental delay with mild elevation of alkaline phosphatase levels

*Kazuyuki Nakamura, †Hitoshi Osaka, ‡Yoshiko Murakami, †Rie Anzai, *Kiyomi Nishiyama, *Hirofumi Kodera, *Mitsuko Nakashima, *Yoshinori Tsurusaki, *Noriko Miyake, ‡Taroh Kinoshita, *Naomichi Matsumoto, and *Hirotomo Saitsu

Epilepsia, 55(2):e13–e17, 2014
doi: 10.1111/epi.12508



Kazuyuki Nakamura is a pediatric neurologist, and researches for epilepsy and brain malformation.

Summary

Aberrations in the glycosylphosphatidylinositol (GPI)–anchor biosynthesis pathway constitute a subclass of congenital disorders of glycosylation, and mutations in seven genes involved in this pathway have been identified. Among them, mutations in *PIGV* and *PIGO*, which are involved in the late stages of GPI-anchor synthesis, and *PGAP2*, which is involved in fatty-acid GPI-anchor remodeling, are all causative for hyperphosphatasia with mental retardation syndrome (HPMRS). Using whole exome sequencing, we identified novel compound heterozygous *PIGO* mutations (c.389C>A [p.Thr130Asn] and c.1288C>T [p.Gln430*]) in two siblings, one of them having epileptic encephalopathy. GPI-anchored proteins (CD16 and CD24) on blood granulocytes were slightly decreased compared with a control and his mother. Our patients lacked the characteristic features of HPMRS, such as facial dysmorphology (showing only a tented mouth) and hypoplasia of distal phalanges, and had only a mild elevation of serum alkaline phosphatase (ALP). Our findings therefore expand the clinical spectrum of GPI-anchor deficiencies involving *PIGO* mutations to include epileptic encephalopathy with mild elevation of ALP.

KEY WORDS: Congenital disorders of glycosylation, Epileptic encephalopathy, Glycosylphosphatidylinositol anchors, *PIGO*.

More than 100 mammalian cell-surface proteins are anchored to the plasma membrane by the addition of glycosylphosphatidylinositol (GPI) to their C-termini. More than 20 genes are involved in the GPI-anchor biosynthesis pathway[1,2] of which 7 are mutated in GPI-anchor deficiencies, a subclass of congenital glycosylation disorders, in association with neurologic impairments.[3–7] Among them, mutations in *PIGV*, *PIGO* (both are involved in the last step of GPI-anchor synthesis), and *PGAP2* (involved in fatty-acid GPI-anchor remodeling) have been identified in patients with hyperphosphatasia with mental retardation syndrome (HPMRS), also known as Mabry syndrome.[3–8]

PIGO encodes GPI ethanolamine phosphate transferase 3, which is also known as phosphatidylinositol-glycan biosynthesis class O. To date, only three HPMRS families with compound heterozygous mutations in *PIGO* have been reported. In this study, we performed whole exome sequencing of a Japanese family containing two affected siblings, one of them having epileptic encephalopathy, and identified novel *PIGO* mutations that expand the clinical spectrum of *PIGO* abnormalities to include epileptic encephalopathy.

Accepted November 6, 2013; Early View publication January 13, 2014.
*Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama, Japan; †Division of Neurology, Clinical Research Institute, Kanagawa Children's Medical Center, Yokohama, Japan; and ‡Research Institute for Microbial Diseases and World Premier International Immunology Frontier Research Center, Osaka University, Osaka, Japan

Address correspondence to Hirotomo Saitsu, Department of Human Genetics, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan. E-mail: hsaitsu@yokohama-cu.ac.jp

METHODS

DNA samples and subjects

All four family members (two affected siblings with epileptic encephalopathy and their parents) were analyzed. Clinical information, peripheral blood samples (individual II-1 and his parents), and the umbilical cord of individual II-2 were obtained after written informed consent was given. DNA was extracted using standard methods. Experimental protocols were approved by the institutional review board of Yokohama City University School of Medicine.

Whole exome sequencing (WES)

Genomic DNA was captured using the SureSelect Human All Exon v4 Kit (51 Mb; Agilent Technologies, Santa Clara, CA, U.S.A.) and sequenced on an Illumina HiSeq2000 (Illumina, San Diego, CA, U.S.A.) with 101 bp paired-end reads. Exome data processing, variant calling, and variant annotation were performed as previously described.[9] *PIGO* mutations detected by WES were confirmed by Sanger sequencing, and searched for in the variant database of our 408 in-house control exomes. For individual II-2, only those *PIGO* mutations identified in individual II-1 were checked by Sanger sequencing.

Flow cytometry

Surface expression of GPI-anchored proteins was examined as previously described.[8]

RESULTS

Clinical features

A summary of the clinical features of individuals II-1 and II-2 is shown in Table S1. Both siblings had intractable seizures and severe developmental delay, which were compatible with epileptic encephalopathy.

Case report 1

Individual II-1 is a 19-year-old male born to nonconsanguineous parents after a 38-week gestation with no asphyxia. His birth weight was 3,250 g (+0.5 standard deviation [SD]), height 52.0 cm (−1.4 SD), and head circumference 34.0 cm (−0.5 SD). Developmental milestones were delayed with no head control achieved at 6 months. At 1 year of age, he developed complex partial seizures with staring, crying, and irregular respiration leading to cyanosis. Brain magnetic resonance imaging (MRI) revealed no abnormalities (Fig. 1A,B). At 1 year and 11 months of age, he had intractable seizures refractory to valproate, zonisamide, and clonazepam. His body weight at this time was 10.54 kg (−0.8 SD), height 84.8 cm (−0.1 SD), and head circumference 45.3 cm (−1.9 SD). He was able to smile but unable to control his head or speak any meaningful words. He had a high arched palate and a tented mouth (Fig. 1E). His muscle tone was hypotonic, and deep tendon reflexes were normal with negative Babinski sign. Chorea was observed mainly in the upper extremities. He did not show brachytelephalangy or nail aplasia (Fig. 1F).

Interictal electroencephalography (EEG), motor conduction velocities, visual evoked potential, short-latency somatosensory evoked potentials, and electroretinogram were normal. Auditory brain responses revealed only wave I. Serum alkaline phosphatase (ALP) levels were 436 U/L (normal range, 145–420),[10] and calcium and phosphate levels were normal. Metabolic analysis including lactate, pyruvate, very long fatty acids, and organic acid showed no abnormalities. His epileptic attacks sometimes led to generalized tonic–clonic seizures. Ictal EEG showed rhythmic fast waves, which appeared at the left side of the central sulcus, followed by diffuse irregular spikes and waves. Phenytoin and bromide treatment slightly decreased the seizure frequency. He was often admitted to the hospital (>40 times) with respiratory insufficiency following upper respiratory tract infection and/or prolonged convulsions, and initiated home oxygen therapy at 2 years of age.

Swallowing and hand movement gradually deteriorated, and spastic quadriplegia and hypertonus with rigidity of both upper and lower limbs appeared at 4 years of age. At 6 years of age, his condition gradually deteriorated, and a brain MRI at 6 years of age revealed diffuse cerebral and cerebellar atrophy (Fig. 1C,D). ALP was slightly elevated at around 10 years of age (900 U/L [normal range 130–560]), followed by a gradual decrease at around the age of 19 (300 U/L [normal range 65–260]). At this time he required mechanical ventilation. He had a very severe intellectual disability and partial seizures with dyspnea every day, despite administration of phenytoin, valproic acid, phenobarbital, bromide, clobazam, and nitrazepam. Pyridoxine has not been administered.

Case report 2

Individual II-2, the younger sister of individual II-1, was born without asphyxia. She did not show any facial dysmorphology or other congenital malformations. At 7 months of age, she developed generalized tonic–clonic seizures for which she was administered phenobarbital. At 1 year of age, she showed developmental delay with no head control. At this time, she was admitted to hospital due to epileptic convulsive status, and she died from multiorgan failure 3 days later. No autopsy was performed.

Identification of *PIGO* mutations and flow cytometry analysis

We filtered out variants registered in dbSNP135 data and our in-house 91 control exomes, and narrowed down 193 rare protein-altering and splice-site variants (Table S2). Among them, we identified compound heterozygous mutations in two genes: *PIGO* (GenBank accession number

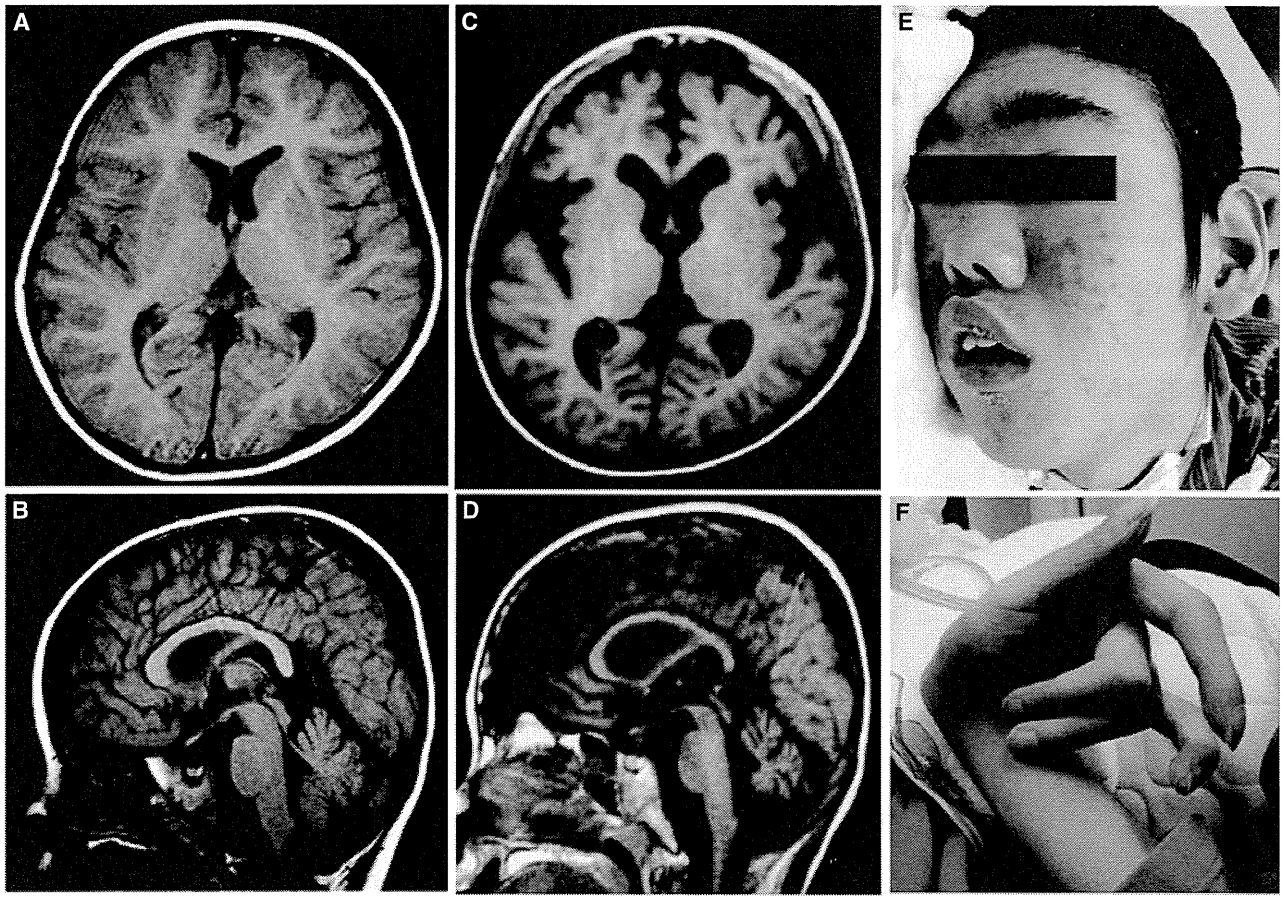


Figure 1.
T_1-weighted brain MRI of individual II-1. Axial (**A**) and sagittal (**B**) images revealed no signal or structural abnormalities at 1 year of age. Axial (**C**) and sagittal (**D**) images at 6 years of age showing diffuse cerebral and cerebellar atrophy. Facial (**E**) and hand (**F**) photographs of individual II-1 at 19 years of age showing tented mouth (**E**) and no anomalous fingers (**F**).

NM_032634.3) and *SCUBE1* (NM_173050.3) (Table S3). No homozygous mutation was detected. Four mutations are rare, but one of two mutations in *SCUBE1* is predicted as a polymorphism by web-prediction tools (Table S3). Therefore, *PIGO* mutations are the primary candidates. Two *PIGO* mutations were also found in his sister (individual II-2). A novel missense mutation c.389C>A (p.Thr130Asn) in exon 1 was inherited from their father and a novel nonsense mutation c.1288C>T (p.Gln430*) in exon 6 was inherited from their mother. Surface expressions of CD16 and CD24 on granulocytes from the individual II-1 were slightly, but clearly, decreased compared with a normal control and his mother, demonstrating GPI-anchor deficiencies in the patient (Fig. S1).

Discussion

In this study, we report two siblings with severe epileptic seizures, developmental delay, and mild elevation of ALP caused by two novel compound heterozygous mutations in *PIGO*. In individuals II-1 and II-2 of the present study, the p.Thr130Asn mutation in *PIGO* is located in an alkaline phosphatase–like core domain, whereas the p.Gln430* mutation is expected to produce a truncated protein that lacks most transmembrane domains (Fig. 2B). To date, only three families with HPRMS are reported in association with compound heterozygous *PIGO* mutations: p.Leu957Phe and p.Thr788Hisfs*5 in the first family, p.Leu957Phe and c.3069+5G>A skipping exon 9 leading to c.2855_3069del (p.Val952Aspfs*24) in the second,[5] and c.355C>T (p.Arg119Trp) and c.2497_2498del (p.Ala834Cysfs*131) in the third.[8] These five mutations led to markedly decreased expression of CD16, CD24, and CD59 on granulocytes from the patient or failed to recover expression of GPI-anchored proteins in *PIGO*-deficient CHO cells, suggesting that expression of GPI-anchored proteins was severely impaired in the patients.[5,8] On the other hand, individual II-1 with p.Thr130Asn and p.Gln430* mutations showed mildly decreased expression of CD16 and CD24 on the surface of blood granulocytes. This difference in the expression of GPI-anchored proteins might be associated with lacking characteristic features of HPMRS in individual

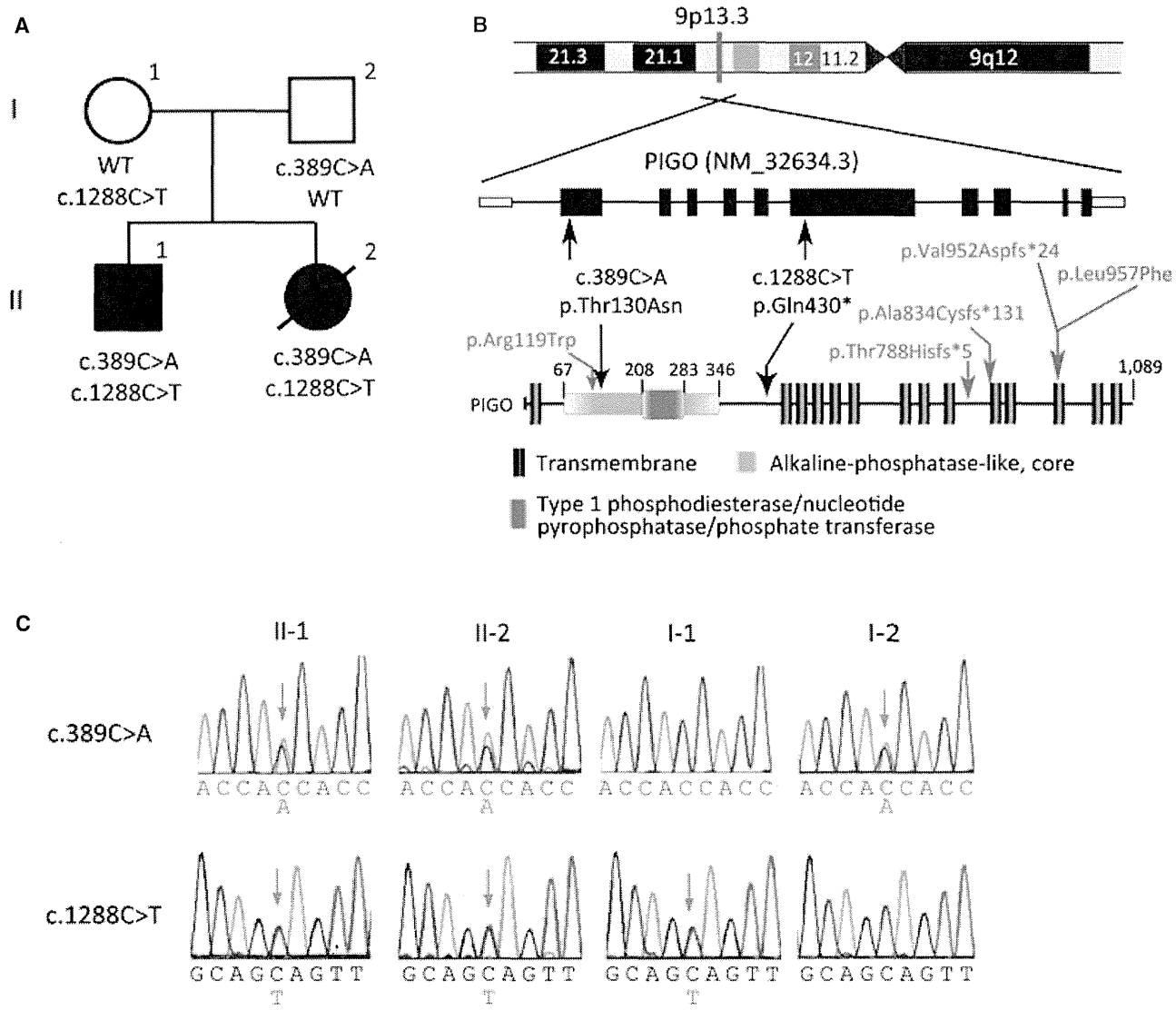


Figure 2.
(**A**) Familial pedigree of individuals I (II-1) and 2 (II-2). (**B**) Distribution of *PIGO* mutations. Previously reported mutations are highlighted in red. (**C**) Individuals II-1 and II-2 carrying compound heterozygous mutations in *PIGO*. Their mother (I-1) carried c.1288C>T (p.Gln430*), and their father (I-2) carried c.389C>A (p.Thr130Asn).

II-1, such as facial dysmorphic features, hypoplasia of distal phalanges, and elevation of serum ALP. Of interest, both patients in our report and a patient reported by Kuki et al. possessed missense mutations commonly in an alkaline phosphatase–like core domain, and showed progressive cerebral and cerebellar atrophy, and more severe intractable epilepsy and developmental delay than the other two families with *PIGO* mutations reported by Krawitz et al.[5,8] This fact raised a possibility that mutations in the alkaline phosphatase–like core domain can affect brain development and function more specifically regardless of expression of GPI-anchored proteins in blood granulocytes. Further accumulation of patients with *PIGO* mutations and functional analysis using neuronal cells are required for elucidating phenotype–genotype correlations in association with *PIGO* mutations.

Our data expand the clinical spectrum of GPI-anchor deficiencies to include epileptic encephalopathy. In addition, it has been recently reported that mutations in the *SLC35A2* encoding UDP-galactose transporter cause a congenital disorder of glycosylation in three patients, and five of them showed seizures with hypsarrhythmia pattern on electroencephalography.[11,12] Therefore, it is likely that abnormalities in glycosylation, including the GPI pathway, may be one of the underlying defects in epileptic encephalopathy.

In conclusion, we have described two siblings with epileptic encephalopathy that harbor novel compound

heterozygous mutations in *PIGO*. Further genetic analysis of GPI-anchor synthesis pathway is needed for the understanding of epileptic encephalopathy.

Acknowledgments

We would like to thank the patients and their families for their participation in this study. We thank Aya Narita and Nobuko Watanabe for technical assistance. This work was supported by the Ministry of Health, Labour and Welfare of Japan; a Grant-in-Aid for Scientific Research (A), (B), and (C) from the Japan Society for the Promotion of Science (A: 24249019, B: 25293085 25293235, C: 23590363); the Takeda Science Foundation; the Japan Science and Technology Agency; the Strategic Research Program for Brain Sciences (11105137); and a Grant-in-Aid for Scientific Research on Innovative Areas (Transcription Cycle, Exploring molecular basis for brain diseases based on personal genomics) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (12024421, 25129705).

Disclosure

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines. None of the authors has any conflicts of interest to disclose.

References

1. Kinoshita T, Fujita M, Maeda Y. Biosynthesis, remodelling and functions of mammalian GPI-anchored proteins: recent progress. *J Biochem* 2008;144:287–294.
2. Fujita M, Kinoshita T. GPI-anchor remodeling: potential functions of GPI-anchors in intracellular trafficking and membrane dynamics. *Biochim Biophys Acta* 2012;1821:1050–1058.
3. Freeze HH, Eklund EA, Ng BG, et al. Neurology of inherited glycosylation disorders. *Lancet Neurol* 2012;11:453–466.
4. Freeze HH. Understanding human glycosylation disorders: biochemistry leads the charge. *J Biol Chem* 2013;288:6936–6945.
5. Krawitz PM, Murakami Y, Hecht J, et al. Mutations in *PIGO*, a member of the GPI-anchor-synthesis pathway, cause hyperphosphatasia with mental retardation. *Am J Hum Genet* 2012;91: 146–151.
6. Krawitz PM, Murakami Y, Riefl A, et al. *PGAP2* mutations, affecting the GPI-anchor-synthesis pathway, cause hyperphosphatasia with mental retardation syndrome. *Am J Hum Genet* 2013; 92:584–589.
7. Hansen L, Tawamie H, Murakami Y, et al. Hypomorphic mutations in *PGAP2*, encoding a GPI-anchor-remodeling protein, cause autosomal-recessive intellectual disability. *Am J Hum Genet* 2013;92: 575–583.
8. Kuki I, Takahashi Y, Okazaki S, et al. Vitamin B6-responsive epilepsy due to inherited GPI deficiency. *Neurology* 2013;81:1467–1469.
9. Saitsu H, Nishimura T, Muramatsu K, et al. *De novo* mutations in the autophagy gene *WDR45* cause static encephalopathy of childhood with neurodegeneration in adulthood. *Nat Genet* 2013;45:445–449.
10. Turan S, Topcu B, Gokce I, et al. Serum alkaline phosphatase levels in healthy children and evaluation of alkaline phosphatase z-scores in different types of rickets. *J Clin Res Pediatr Endocrinol* 2011;3: 7–11.
11. Ng BG, Buckingham KJ, Raymond K, et al. Mosaicism of the UDP-galactose transporter *SLC35A2* causes a congenital disorder of glycosylation. *Am J Hum Genet* 2013;92:632–636.
12. Kodera H, Nakamura K, Osaka H, et al. *De novo* mutations in *SLC35A2* encoding a UDP-galactose transporter cause early-onset epileptic encephalopathy. *Hum Mutat* 2013;34:1708–1714.

Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Expression of GPI-anchored proteins on granulocytes from the patient.

Table S1. Clinical features of individuals with *PIGO* mutations.

Table S2. Summary of the exome sequencing performance.

Table S3. Candidate variants corresponding to the autosomal recessive model.