more than a coincidence and reflective of a shared mechanism and genetic etiology.

If the lesions in our families are caused by genetic mutations giving rise to increased susceptibility, then the question remains as to how different family members may have different lesions, especially in families 2, 3, 4, and 6 in which the proband has FCD but their relative has a developmental tumor or hemimegalencephaly. In TSC, there is significant phenotypic pleiotropy with variation of lesions in family members with the same mutation. More so, within an individual patient with TSC, a mutation can result in both a dysplastic cortical lesion (cortical tubers) and a benign neoplastic lesion (giant cell astrocytoma). Alternatively, lesions in these families could result from "two hits," one causing a nonpathogenic germ line mutation in a cortical development gene carried within the family, and the other causing a somatic mutation of the other allele of that gene within the FCD or other MCD. This hypothesis may explain the sparing of other family members. One may speculate that the nature of the "second hit" may explain the differences in phenotypes within families, with the second mutation either affecting different cellular precursors (i.e., glial vs. neuronal) or affecting precursors at different developmental time points (i.e., early vs. late progenitors).

Identifying the etiology of FCD and related lesions has remained elusive, despite FCD being a relatively common entity. The six families presented herein provide suggestive clinical evidence of a genetic link between FCD, gangliglioma, hemimegalencephaly, and DNET. It must be acknowledged that FCD and related lesions may occur within the same pedigree as a chance association. Although accurate data on the prevalence of FCD are lacking, we estimate from our own experience that FCD and related lesions are the cause of epilepsy in at most 1 in 100 patients, so the occurrence in multiple families by chance alone would be quite unlikely. Families 1 and 6 in our study, each with siblings with pathologic features of FCD type IIa, are therefore the most convincing pedigrees supporting our hypothesis of a shared genetic susceptibility. Whether this susceptibility extends to other pedigrees that include a family member with FCD and others with MRI-negative epilepsy requires further study, but it is important that in such families the MRI studies of patients with MRI-negative epilepsy be closely scrutinized for subtle lesions. Exploring these relationships further will require molecular exploration of germline mutations in families of interest, in combination with the study of tissue resected at epilepsy surgery for somatic mutations.

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Disclosures or Conflicts of Interest

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

References

1. Blümcke I, Thom M, Aronica E, et al. The clinicopathologic spectrum of focal cortical dysplasias: a consensus classification proposed by an ad hoc Task Force of the ILAE Diagnostic Methods Commission. *Epilepsia* 2011;52:158–174.
2. Barkovich AJ, Guerrini R, Kuzniecky RI, et al. A developmental and genetic classification for malformations of cortical development: update 2012. *Brain* 2012;135:1348–1369.
3. Strauss KA, Puffenberger EG, Huentelman MJ, et al. Recessive symptomatic focal epilepsy and mutant contactin-associated protein-like 2. *N Engl J Med* 2006;354:1370–1377.
4. Hasselblatt M, Kurlemann G, Rickert CH, et al. Familial occurrence of dysembryoplastic neuroepithelial tumor. *Neurology* 2004;62:1020–1021.
5. Chen J, Tsai V, Parker WE, et al. Detection of human papillomavirus in human focal cortical dysplasia type IIB. *Ann Neurol* 2012;72:881–892.
6. Poduri A, Evrony GD, Cai X, et al. Somatic activation of AKT3 causes hemispheric developmental brain malformations. *Neuron* 2012;74:41–48.
7. Lee JH, Huynh M, Silhavy JL, et al. De novo somatic mutations in components of the PI3K-AKT3-mTOR pathway cause hemimegalencephaly. *Nat Genet* 2012;44:941–945.
8. Crino PB. Focal brain malformations: seizures, signaling, sequencing. *Epilepsia* 2009;50(Suppl. 9):3–8.
9. Saad AG, Jayarao M, Chin LS, et al. Ganglioglioma associated with cerebral cortical dysplasia: an unusual case with extensive leptomeningeal involvement. *Pediatr Dev Pathol* 2008;11:474–478.
10. Takahashi A, Hong SC, Seo DW, et al. Frequent association of cortical dysplasia in dysembryoplastic neuroepithelial tumor treated by epilepsy surgery. *Surg Neurol* 2005;64:419–427.
11. Prayson RA. Composite ganglioglioma and dysembryoplastic neuroepithelial tumor. *Arch Pathol Lab Med* 1999;123:247–250.
12. Ljungberg MC, Bhattacharjee MB, Lu Y, et al. Activation of mammalian target of rapamycin in cytomegalic neurons of human cortical dysplasia. *Ann Neurol* 2006;60:420–429.
13. Samadani U, Judkins AR, Akpalu A, et al. Differential cellular gene expression in ganglioglioma. *Epilepsia* 2007;48:646–653.
14. Aronica E, Boer K, Baybis M, et al. Co-expression of cyclin D1 and phosphorylated ribosomal S6 proteins in hemimegalencephaly. *Acta Neuropathol* 2007;114:287–293.
15. Gumbinger C, Rohsbach CB, Schulze-Bonhage A, et al. Focal cortical dysplasia: a genotype–phenotype analysis of polymorphisms and mutations in the TSC genes. *Epilepsia* 2009;50:1396–1408.

Supporting Information

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

Figure S1. Selected brain MRI and neuropathology images of families 2, 3, 4, and 5.

PIGA mutations cause early-onset epileptic encephalopathies and distinctive features

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ABSTRACT

Objective: To investigate the clinical spectrum caused by mutations in *PIGA* at Xp22.2, which is involved in the biosynthesis of the glycosylphosphatidylinositol (GPI) anchor, among patients with early-onset epileptic encephalopathies (EOEEs).

Methods: Whole-exome sequencing was performed as a comprehensive genetic analysis for a cohort of 172 patients with EOEEs including early myoclonic encephalopathy, Ohtahara syndrome, and West syndrome, and *PIGA* mutations were carefully investigated.

Results: We identified 4 *PIGA* mutations in probands showing early myoclonic encephalopathy, West syndrome, or unclassified EOEE. Flow cytometry of blood granulocytes from patients demonstrated reduced expression of GPI-anchored proteins. Expression of GPI-anchored proteins in *PIGA*-deficient JY5 cells was only partially or hardly restored by transient expression of PIGA mutants with a weak TATA box promoter, indicating a variable loss of PIGA activity. The phenotypic consequences of *PIGA* mutations can be classified into 2 types, severe and less severe, which correlate with the degree of PIGA activity reduction caused by the mutations. Severe forms involved myoclonus and asymmetrical suppression bursts on EEG, multiple anomalies with a dysmorphic face, and delayed myelination with restricted diffusion patterns in specific areas. The less severe form presented with intellectual disability and treatable seizures without facial dysmorphism.

Conclusions: Our study confirmed that *PIGA* mutations are one genetic cause of EOEE, suggesting that GPI-anchor deficiencies may be an underlying cause of EOEE. ***Neurology*® 2014;82:1587–1596**

GLOSSARY

ADC = apparent diffusion coefficient; **cDNA** = complementary DNA; **DWI** = diffusion-weighted image; **EME** = early myoclonic encephalopathy; **EOEE** = early-onset epileptic encephalopathy; **GPI** = glycosylphosphatidylinositol; **GPI-AP** = glycosylphosphatidylinositol-anchored protein; **OS** = Ohtahara syndrome; **WES** = whole-exome sequencing.

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Early-onset epileptic encephalopathies (EOEEs) present with developmental impairment and disastrous seizures starting in early infancy with a mode of age dependency. Ohtahara syndrome (OS) and early myoclonic encephalopathy (EME), both of which show a distinctive EEG finding called suppression-burst pattern, are neonatal EOEEs. Genetic approaches have revealed some of the genes that are mutated in EOEEs. For instance, *ARX*, *STXBP1*, *CASK*, *KCNQ2*, and *SCN2A* are mutated in OS,[1–5] while *ARX*, *CDKL5*, and *SPTAN1* mutations cause West syndrome or infantile spasms.[6–8]

Mutations in 8 genes (*PIGA*, *PIGM*, *PIGN*, *PIGV*, *PIGL*, *PIGO*, *PIGT*, and *PGAP2*) involved in the biosynthesis of the glycosylphosphatidylinositol (GPI) anchor, a glycolipid structure embedded in the plasma membrane that attaches to hundreds of cell-surface proteins, have been identified in patients with a variety of multiple congenital anomalies, intellectual disability, and epileptic seizures.[9–16] Somatic mutations of *PIGA* at Xp22.2, which is involved in

Supplemental data at Neurology.org

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the first step of the GPI biosynthesis, are responsible for paroxysmal nocturnal hemoglobinuria, and its germline mutation, were recently identified in a family with multiple congenital anomalies, neonatal seizures, and a poor prognosis.[11] At least 2 of 3 patients in this family showed severe myoclonic seizures with suppression bursts on EEG, strongly suggesting EME. The known mutations in EME prompted us to investigate *PIGA* in the EOEE patient cohort including EME and OS. We identified *PIGA* mutations in 5 patients from 4 families with EOEEs and present the clinical phenotypes of the patients and functional effects of the mutations in this study.

METHODS Patients. A total of 172 patients with EOEEs (2 with EME, 50 with OS, 50 with West syndrome or infantile spasms, 7 with malignant migrating partial seizures in infancy, and 63 with unclassified epileptic encephalopathy with an age at onset of <1 year; 90 male and 82 female patients) were analyzed by whole-exome sequencing (WES), and *PIGA* mutations were carefully investigated using WES data.

Patients had been mainly enrolled in the Japanese collaborative study for EOEE since 2003. The diagnosis was made based on clinical features and characteristic EEG patterns. Patients with mutations in *STXBP1, ARX, KCNQ2, SCN1A, SCN2A, KCNT1, CDKL5, CASK,* or *MECP2,* which were detected by high-resolution melting analysis, target capture analysis, direct sequencing analysis, or WES, were excluded from the study.

Whole-exome sequencing. Patient and parental genomic DNA was obtained from peripheral blood leukocytes using standard methods. DNA was captured using the SureSelectXT Human All Exon Kit (v4 or v5; Agilent Technologies, Santa Clara, CA) and sequenced on an Illumina HiSeq2000 (Illumina, San Diego, CA) with 101–base pair paired-end reads. Image analysis and base calling were performed using sequence control software real-time analysis and CASAVA software v1.8 (Illumina). Exome data processing, variant calling, and variant annotation were performed as previously described.[17–19] All novel mutations in *PIGA* were verified using Sanger sequencing.

Fluorescence-activated cell sorting analysis. Surface expression of GPI-anchored proteins (GPI-APs) was determined by staining cells with Alexa 488–conjugated inactivated aerolysin (FLAER; Protox Biotech, Victoria, Canada) and appropriate primary antibodies, namely, mouse anti-CD59 (5H8), DAF (IA10), CD16 (3G8), CD24 (ML5), and CD48 (BJ40), followed by a phycoerythrin-conjugated anti-mouse immunoglobulin G antibody (3G8, ML5, BJ40, and secondary antibodies; BD Biosciences, Franklin Lakes, NJ). Cells were analyzed by flow cytometry (Cant II; BD Biosciences).

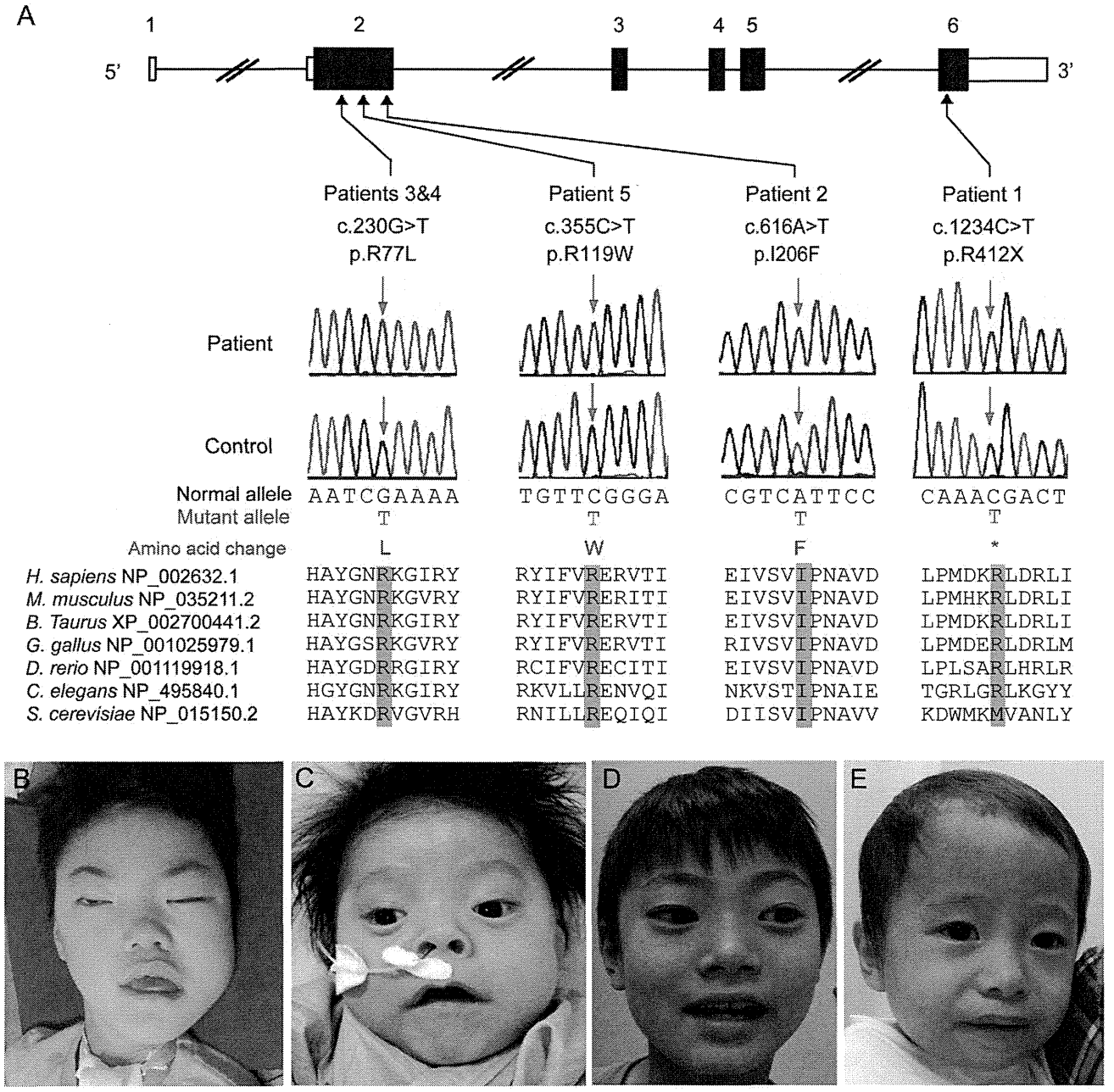
Functional analysis using *PIGA*-deficient B lymphoblastoid cells (JY5). FLAG-tagged human *PIGA* complementary DNA (cDNA) and mutant cDNAs, generated by site-directed mutagenesis, were subcloned into the pMEoriP vector, a strong promoter (SRα)-driven vector or pTAoriP, a weak TATA box promoter-driven vector. Plasmid DNA was transfected by electroporation into *PIGA*-deficient JY5 cells. Expression of GPI-APs was analyzed by fluorescence-activated cell sorting. PIGA protein levels in transfected cells were determined by Western blotting using an anti-FLAG antibody (M2; Sigma, St. Louis, MO).

Standard protocol approvals, registrations, and patient consents. The experimental protocols were approved by the institutional review boards for ethical issues of Yamagata University Faculty of Medicine, Yokohama City University School of Medicine, and Osaka University, Japan. Written informed consent was obtained from all individuals and/or their families in compliance with relevant Japanese regulations. Permission for publishing photographs was also obtained from the parents.

RESULTS Identification of *PIGA* mutations. No mutations were found in *SLC25A22,* which had been reported in a family of EME.[20] We identified 4 hemizygous *PIGA* mutations in 3 sporadic patients and 2 siblings with EOEE. One mutation (c.1234C>T [p.R412X]) had previously been reported,[11] while the other 3 were novel missense mutations (c.230G>T [p.R77L], c.616A>T [p.I206F], and c.355C>T [p.R119W]). DNA from the mother of patient 1 (p.R412X) was unavailable. Three missense mutations were maternally inherited. All mutations were absent from the 6,500 exomes of the National Heart, Lung, and Blood Institute exome project and our 573 in-house control exomes (281 male and 292 female patients). All 4 mutations occurred at evolutionarily conserved amino acids (figure 1A) and were predicted to be highly damaging to the protein structure by SIFT, PolyPhen-2, and MutationTaster (table e-1 on the *Neurology*® Web site at Neurology.org), which supported their pathogenicity.

Clinical features of patients with the *PIGA* mutation. The clinical information of individuals with a *PIGA* mutation is summarized in table 1, and their facial appearances and representative brain images are shown in figures 1 and 2, respectively. EEG findings (figure e-1) and detailed case reports (appendix e-1) are available in supplemental data. Two patients were associated with polyhydramnios. Birth weight and length were normal in 3 patients (patients 2, 3, and 5) who were born at term, but the other 2 who were born at preterm showed higher (patient 1) or lower (patient 4) birth weights than normal. Three patients with the severe phenotype (patients 1, 2, and 5) showed facial dysmorphisms (figure 1, B and C), including a depressed nasal bridge, short anteverted nose, downturned corners of the mouth, and high arched palate. Patient 1 also showed bilateral vesicoureteral reflux of the most severe grade V. In addition, brain MRI demonstrated a thin corpus callosum and delayed myelination in these patients. Of interest, abnormally high signals on diffusion-weighted images (DWIs) and low signals on the apparent diffusion coefficient (ADC) map at the brainstem, basal ganglia, thalamus, and deep white matter were found in patients 1, 2, and 5 (figure 2, A–D and M–Q). By

Figure 1 *PIGA* mutations in patients with epileptic encephalopathy and dysmorphic features



(A) Schematic presentation of *PIGA* genomic structure. Mutations are indicated based on the transcript variant 1 (GenBank accession number, NM_002641.3). Untranslated regions and coding regions are shown as white and black rectangles, respectively. All mutations occurred at evolutionarily conserved amino acids. Orthologous sequences were aligned using the CLUSTALW Web site. (B–E) Facial appearance of patients 2, 3, 4, and 5. Both patients 2 (B) and 5 (C) show distinct facial features, such as upslanting palpebral fissures, depressed nasal bridge, short anteverted nose, triangular mouth with downturned corners, and high arched palate, compared with patients 3 (D) and 4 (E) with no dysmorphic facial features.

contrast, 2 brothers with a less severe phenotype (patients 3 and 4) showed neither dysmorphic signs nor abnormalities in brain MRI (figure 2, E–L).

The first seizures started between 1 and 7 months of age, and tonic or myoclonic seizures occurred in all patients. Seizures of patients 1, 2, and 5 were refractory to antiepileptic medications, but topiramate was effective for the seizures of patient 3. The initial EEG showed a suppression burst in patient 1; patients 2 and 5 first demonstrated hypsarrhythmia, followed by a symmetrical or asymmetrical suppression burst later (figure e-1). Serum alkaline phosphatase levels were elevated in patients 2 and 5. No patients showed anemia or hemoglobinuria. All patients showed profound intellectual disability, and patients 1, 2, and 5 were bedridden with severe motor disturbance.

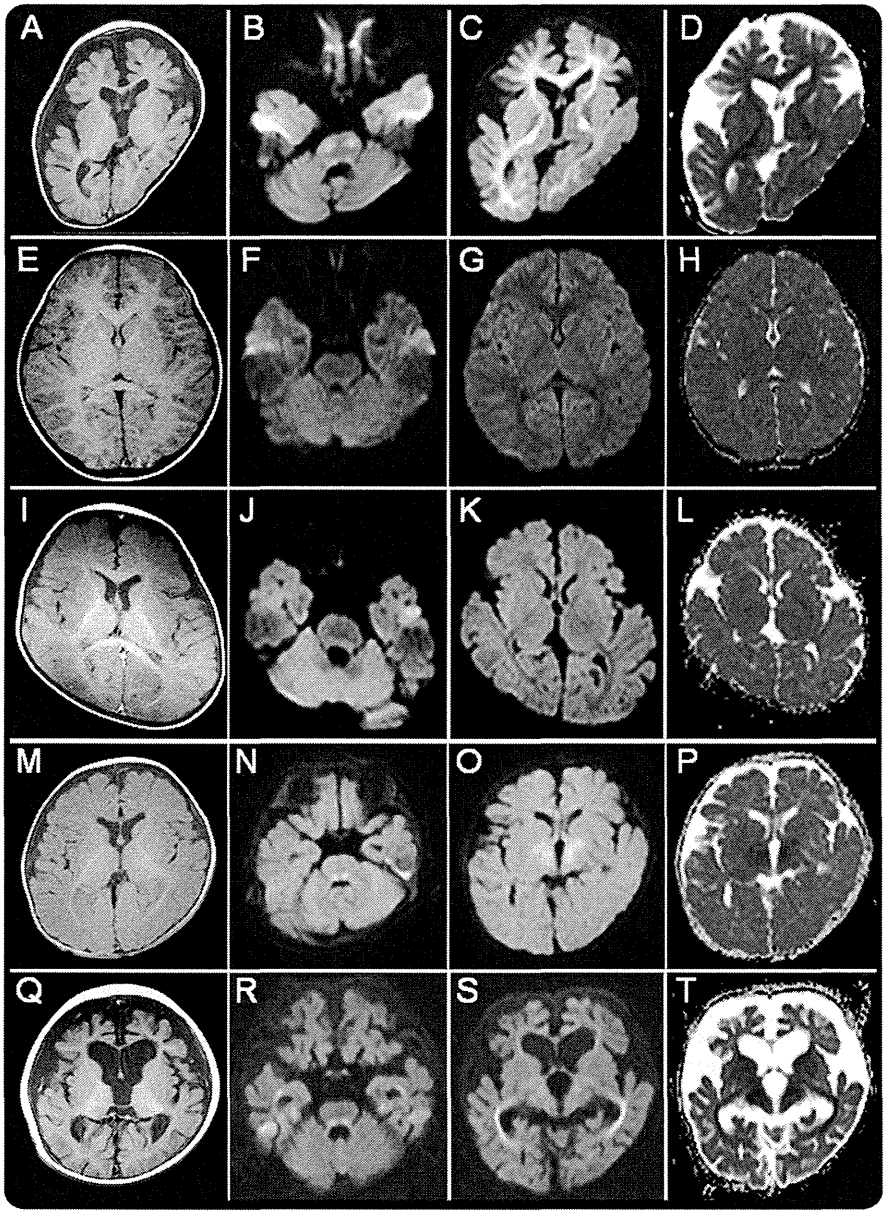
Flow cytometry. We analyzed the surface expression of various GPI-APs on patient granulocytes using flow

Table 1 **Clinical summary of patients with a *PIGA* mutation**

	Patients						
	IV-2	IV-4	1	2	3	4	5
Familial or sporadic	Familial or sporadic	Familial (brother)	Sporadic	Sporadic	Familial (proband)	Familial (brother)	Sporadic
Mutation	c.1234C>T (p.R412X)	c.1234C>T (p.R412X)	c.1234C>T (p.R412X)	c.616A>T (p.I206F)	c.230G>T (p.R77L)	c.230G>T (p.R77L)	c.355C>T (p.R119W)
Current age	Died at 11 wk	Died at 10 wk	6 y	10 y	8 y	18 mo	15 mo
Sex	M	M	M	M	M	M	M
Clinical diagnosis			Ohtahara syndrome, early myoclonic encephalopathy, Schinzel-Giedion syndrome	West syndrome with hypomyelination	Early-onset epileptic encephalopathy	Early-onset epileptic encephalopathy	West syndrome
Polyhydramnios	–	+	+	–	–	–	+
Gestation, wk	Full term	35	33	40	38	36	39
Birth weight, g	3,540	3,500	2,857	3,566	2,715	1,896	3,468
Birth length, cm	53.5	48	42.0	50	50	ND	47
Birth head circumference, cm	37	35.5	33.2	ND	32.5	ND	33.5
Facial dysmorphism	+	+	+	+	–	–	+
Vesicoureteral reflux	+	ND	+	ND	–	–	ND
Joint contractures	+	+	+	+	–	–	–
Hypotonia	+	+	+	–	–	–	+
Hyperreflexia	+	+	ND	–	–	–	+
Seizure onset	Neonate	Neonate	1 mo	3 mo	7 mo	7 mo	3 mo
Seizure types	Myoclonic	Severe myoclonic	Tonic seizures followed by frequent myoclonus	Myoclonus or epileptic spasm-like movement	Tonic seizures, secondarily generalized seizures	Tonic or clonic	Myoclonic seizures, tonic spasms
EEG findings	Suppression burst	Suppression burst	Suppression burst at neonatal period	Hypsarrhythmia at 3 mo, periodic bursts of multifocal epileptic discharges similar to suppression-burst pattern at 10 y	Normal at 7 mo, irregular spike and slow wave and multifocal spikes at 2 and 5 y	Normal at 7 mo	Hypsarrhythmia at 3 mo, suppression burst at 5 mo
Seizure prognosis	Intractable	Intractable	Intractable	Intractable	Seizure-free at 3 y with TPM	Seizure-free at 15 mo	Intractable
Development	Early death	Early death	Hypotonic quadriplegia, profound intellectual disability	Spastic quadriplegia, profound intellectual disability	Profound intellectual disability with autism, but no motor disturbance	Moderate intellectual disability, but no motor disturbance	Hypotonic quadriplegia, profound intellectual disability
Thin corpus callosum	+	+	+	+	–	–	+ (at 9 mo)
White matter immaturity	+	+	+	+	–	–	+ (at 9 mo)
Restricted diffusion pattern	ND	ND	+	+	–	–	+
Elevated serum alkaline phosphatase	ND	+	ND	+	–	–	+

Abbreviations: ND = not determined; TPM = topiramate.

Figure 2 **Brain MRIs of patients with *PIGA* mutations**

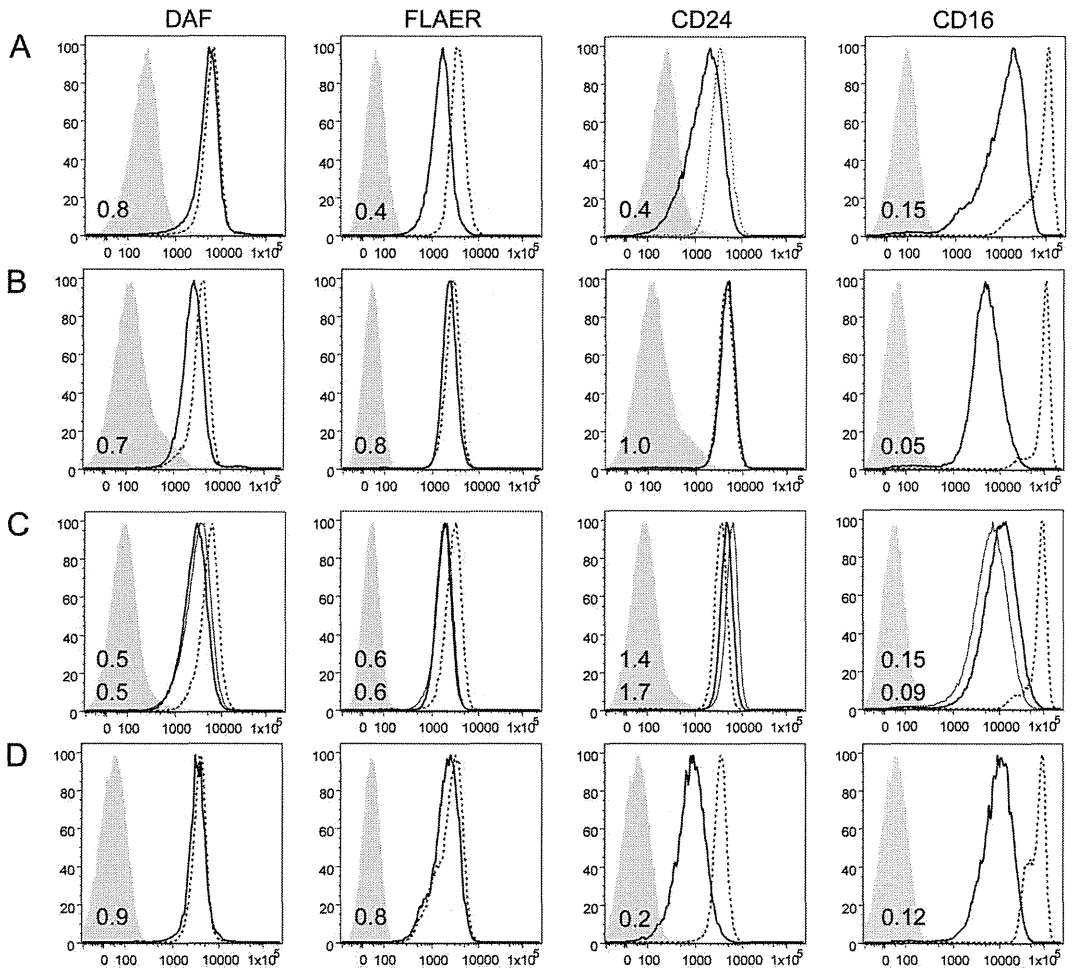


MRIs of patient 2 at 6 months (A) and 7 years (B–D), patient 3 at 3 years (E–H), patient 4 at 7 months (I–L), and patient 5 at 3 months (M–P) and 9 months (Q–T) of age. Left panels (A, E, I, M, Q) show axial T1-weighted images, the 2 middle panels (B, C, F, G, J, K, N, O, R, S) show axial diffusion-weighted images (DWIs), and right panels (D, H, L, P, T) show apparent diffusion coefficient (ADC) maps. Patient 2 and patient 5 at 9 months show cortical atrophy and enlarged ventricles. Note the high signals on DWI in the pontine tegmentum and deep white matter, particularly the optic radiation, of patients 2 and 5 in accordance with their age. The ADC map demonstrated decreased ADC within the same lesion. Patients 3 and 4 show normal images.

cytometry (figure 3). In all 5 patients, the surface expression of CD16 was severely decreased (from 5% to 15% of normal levels). Patient 1, with the most severe clinical symptoms, had a tendency to show reduced expression of other GPI-APs, such as CD24 and FLAER (figure 4A). Because *PIGA* is an X-linked gene and one allele is inactivated during early embryogenesis in female patients, patient mothers would be functionally mosaic for GPI-AP expression. Granulocytes from the mother of patients 3 and 4 showed a significantly decreased expression of CD16 (figure e-2, upper panels), whereas those from the mother of patient 5 showed normal expression (figure e-2, lower panels). The mothers appeared to have no neurologic disorder, suggesting that GPI-sufficient cells may preferentially proliferate in the brain during early embryogenesis.

Functional analysis. *PIGA* cDNAs bearing patient mutations were functionally analyzed by transfecting them into *PIGA*-deficient B lymphoblastoid cells (JY5) and measuring the surface expression of GPI-APs.

Figure 3 Flow cytometry of granulocytes



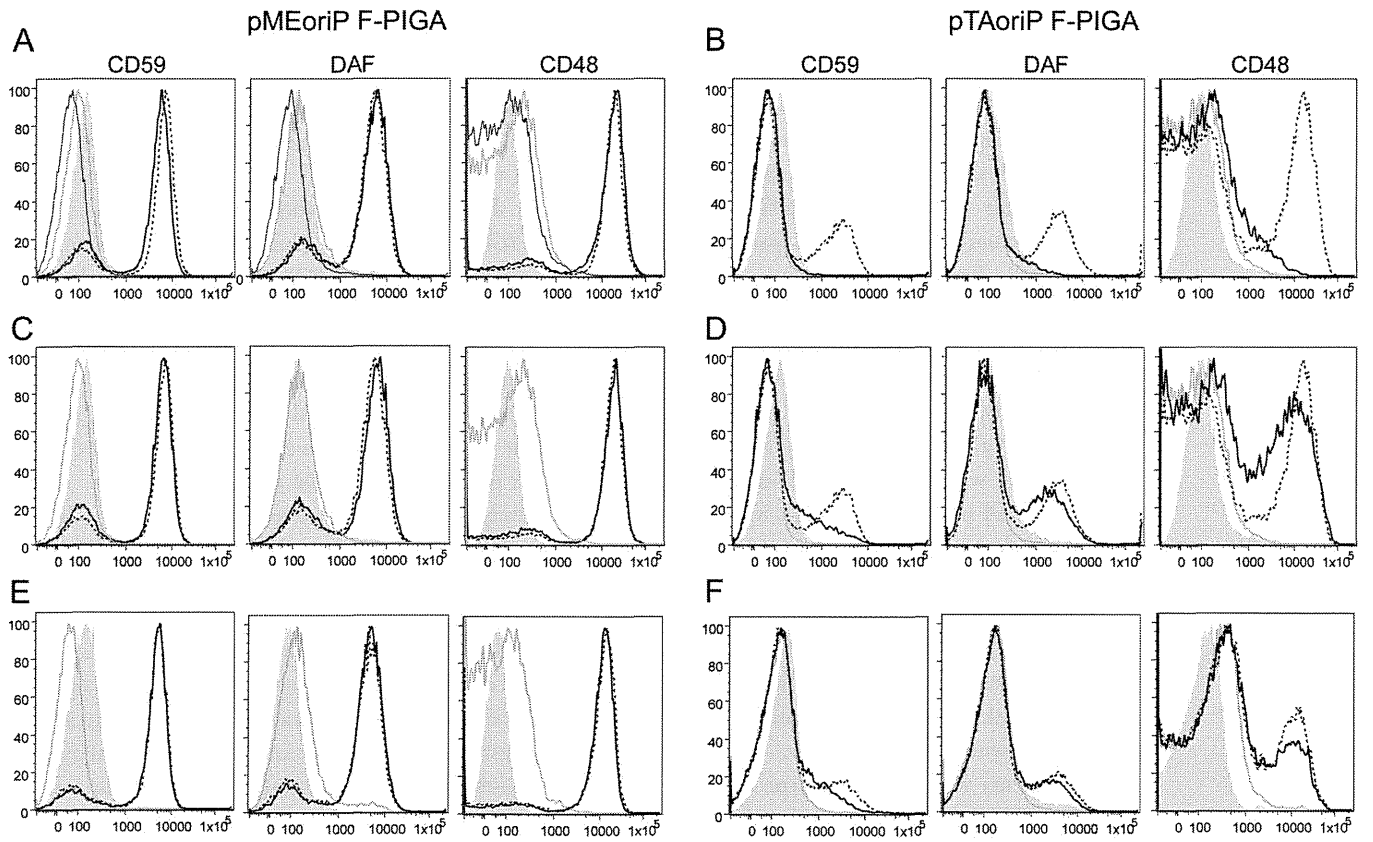
Flow cytometry of patient 1 (R412X) (A), patient 2 (I206F) (B), patients 3 and 4 (2 brothers, R77L) (C), and patient 5 (R119W) (D). In all families, the surface expression of various glycosylphosphatidylinositol-anchored proteins on patient granulocytes (solid lines; patients 3 and 4 are shown in C as thin and thick lines, respectively) was severely decreased compared with the normal control (dotted lines). Light shadows represent isotype controls. Mean fluorescent intensities of each sample against a normal control are shown in each panel (upper, patient 4; lower, patient 3 in C).

When strong promoter (SRα)-driven constructs were used, R412X mutant cDNA completely restored the surface expression of CD59, DAF, and CD48, whereas R412 truncated cDNA had no activity (figure 4A), suggesting that a small amount of full-length PIGA protein was generated by readthrough of a stop codon. When weak promoter-driven constructs (pTA) were used instead, R412X cDNA could not restore the surface expression of GPI-APs, whereas it was completely restored by wild-type cDNA (figure 4B). Similarly, the strong promoter (SRα)-driven I206F and R77L mutant PIGAs completely restored the surface expression of GPI-APs, whereas the weak promoter-driven mutant constructs only partially restored this (figure 4, C–F). Levels of expressed mutant PIGA proteins were similar to or even higher than wild-type levels (figure e-3). A faint band representing full-length PIGA protein harboring R412X could be detected (figure e-3, lane 4), which was consistent with the functional analysis. From these results, we concluded that these mutations affect the PIGA activity leading to inherited GPI deficiency.

DISCUSSION We have identified 4 *PIGA* mutations in 172 probands from a variety of EOEE-affected families, such as EME (n = 1), West syndrome (n = 2), and unclassified EOEE in a sibling. Myoclonus and suppression burst on EEG were recognized in 2 patients with West syndrome and the patient with EME in our cohort, as well as the previously reported family.[11] Indeed, myoclonus and suppression burst on EEG appear to be characteristic features for patients with a *PIGA* mutation.

Other clinical features such as polyhydramnios, facial dysmorphism, joint contractures, hypotonia, and severe developmental delay are recurrently seen in patients with *PIGA* mutations. A previous report of 3 patients with the same nonsense mutation,

Figure 4 Functional analysis of the mutant PIGA



JY5 cells were transiently transfected with pMEoriP (strong SRα promoter-driven, Epstein-Barr [EB] virus origin-containing vector) (panels A, C, and E) or pTAoriP F-PIGA (weak TATA box promoter-driven, EB virus origin-containing vector) (panels B, D, and F) bearing various FLAG-tagged PIGA complementary DNAs. Restoration of the surface expression of CD59, DAF, and CD48 was assessed 2 days later by flow cytometry. Dotted lines represent wild-type PIGA, thick lines represent mutant PIGA, thin lines represent truncated PIGA, and shadows represent isotype controls. (A) Strong promoter-driven R412X PIGA (thick lines) completely rescued the expression of glycosylphosphatidylinositol-anchored proteins (GPI-APs) similar to wild-type PIGA (dotted lines), whereas R412-truncated PIGA (thin lines) had no activity. (B) Weak promoter-driven R412X PIGA (thick lines) did not rescue the surface expression of GPI-APs, whereas wild-type PIGA (dotted lines) did. (C) Strong promoter-driven I206F PIGA (thick lines) completely rescued the expression of GPI-APs similar to wild-type PIGA (dotted lines). (D) Weak promoter-driven I206F PIGA (thick lines) did not rescue the surface expression of GPI-APs, whereas wild-type PIGA (dotted lines) did. (E) Strong promoter-driven R77L PIGA (thick lines) completely rescued the expression of GPI-APs similar to wild-type PIGA (dotted lines). (F) Weak promoter-driven R77L PIGA (thick lines) did not rescue the surface expression of CD59, whereas wild-type PIGA (dotted lines) did.

R412X,[11] as patient 1 in our cohort showed similar or more severe clinical features, such as a large occipitofrontal circumference at birth, early-onset intractable seizures, and severe respiratory failure leading to early death or mechanical ventilation. Complete disruption of the *PIGA* gene results in early embryonic lethality in male mice, while heterozygous female mice have late embryonic lethality, insufficient closure of the neural tube, and a cleft palate.[21] In the present study, a reduced but definite expression of GPI-APs in the granulocytes of patients with R412X and a complete restoration of GPI-AP surface expression by the transfection of R412X mutant cDNA under the control of a strong promoter suggest that small amounts of full-length PIGA protein were generated by the read-through of a stop codon because the cDNA truncated at R412 showed no activity.

The siblings with the *PIGA* p.R77L mutation demonstrated milder clinical symptoms compared with patients with other *PIGA* mutations. They showed neither dysmorphisms nor severe motor disturbance, the onset of their seizures was relatively late, and the findings of their initial EEG and brain MRI were normal. Flow cytometry only revealed a decreased expression of CD16, which contrasts with the more severe phenotype of patient 1 and associated decreased levels of CD16, FLAER, and CD24. According to the functional study using *PIGA*-deficient B lymphoblasts transfected with a weak promoter-driven mutant *PIGA*, the activity of the R77L mutant was higher than that of other mutants. Thus, the phenotype severity appears to correlate with genotype and the residual functional activity of the PIGA protein.

Patients 2 and 5 showed peculiarly high signals on DWI at the specific areas of the brainstem, basal ganglia, thalamus, and deep white matter, particularly the optic radiation as previously reported in patient 1.[22] Although delayed myelination and the volume loss of

white matter including a thin corpus callosum, mild brain atrophy, and mild cerebellar hypoplasia are frequently seen in patients with mutations in other genes involved in the biosynthesis of the GPI anchor, such as *PIGN, PGAP2, DPM1,* and *DPM2,*[10,15,23,24] high signals on DWI have never been reported. In addition, the ADC map showed adversely low or decreased signals, suggesting restricted water diffusion. This pattern (a high DWI signal and low ADC values) can be seen in patients with specific metabolic disorders, such as nonketotic hyperglycinemia, phenylketonuria, maple syrup urine disease, Leigh encephalopathy, infantile neuroaxonal dystrophy, Wilson disease, metachromatic leukodystrophy, and Canavan disease.[25] Indeed, metabolic disorders, particularly nonketotic hyperglycinemia, are strongly associated with EME, which is common in patients with *PIGA* mutations. A brain MRI of a patient in early infancy with a recently reported *PIGO* deficiency also showed hypomyelination and abnormally high signals in T2-weighted images from the bilateral basal ganglia to the brainstem.[26] While the pathologic mechanism for restricted diffusion patterns in specific areas is unknown, this finding may be useful to screen patients with a GPI deficiency.

Patients with the severe type of *PIGA* mutation showed both an asymmetrical and symmetrical pattern of suppression burst on EEG in this study. The suppression burst pattern is characteristic for 2 types of EOEE, OS and EME, and most patients of both disorders show a symmetrical pattern. The asymmetrical pattern has been reported in patients with agenesis of the corpus callosum such as Aicardi syndrome,[27] and *KCNQ2* mutations.[4] All 3 patients with the asymmetrical suppression burst in the present study also showed white matter immaturity with a thin corpus callosum and abnormally high signals in deep white matter on DWI. These data indicate a disturbed connectivity of the bilateral hemisphere in patients with *PIGA* mutations. The adverse advancement of the EEG findings from hypsarrhythmia to suppression burst in our cases, which is usually observed in neonates, might reflect the retrogression of brain function, which is also seen in the progression of brain atrophy.

Patient 1 showed severe hydronephrosis caused by the vesicoureteral reflux and hepatoblastoma, so a diagnosis of Schinzel-Giedion syndrome was made. This is an autosomal dominant disorder characterized by severe developmental delay, distinctive facial features with a prominent forehead, midface retraction, short, upturned nose, and either hydronephrosis or typical skeletal malformations, such as sclerotic skull base, wide occipital synchondrosis, increased cortical density or thickness, and broad ribs.[28] *SETBP1* mutations have been reported in patients with Schinzel-Giedion syndrome[29] but were not identified in our patient. Because of the phenotypic similarities between patients with *PIGA* mutation and those with Schinzel-Giedion syndrome, we suggest that patients with Schinzel-Giedion syndrome with no *SETBP1* mutations should undergo genetic analysis of their *PIGA* gene or other genes involved in the biosynthesis of the GPI anchor.

Patients with mutations in *PIGL, PIGM, PIGN, PIGO, PIGT, DPM2,* and *MPDU1* often die in early childhood.[9,12,14–16,23,30] While pneumonia is the main cause of death in these patients, intractable seizures, which rigorously worsen the prognosis of life expectancy and cognitive function, frequently occur. It is of interest that the targeted agents butyrate and pyridoxine were reported to be effective for seizure treatment in patients with *PIGM* or *PIGO* mutation, respectively.[26,31] However, patient 5 in this study did not respond to pyridoxine. The study of more patients will facilitate the establishment of personalized treatment methods for patients with GPI deficiencies.

Our study demonstrated that mutations in *PIGA* are causative for a variety of EOEEs, particularly for patients with myoclonus and asymmetrical suppression burst on EEG. Multiple anomalies with facial dysmorphism resembling Schinzel-Giedion syndrome, delayed myelination with restricted diffusion patterns at the brainstem, and deep white matter are key findings in a severe form in patients with *PIGA* mutations. Nevertheless, a wide range of clinical phenotypes of *PIGA* mutations should be kept in mind, including the less severe forms involving intellectual disability and treatable seizures without facial dysmorphism.

AUTHOR CONTRIBUTIONS

Mitsuhiro Kato: study concept and design, analysis of the clinical data, interpretation of the data, and drafting/revising of the manuscript. Hirotomo Saitsu: study concept and design, analysis of the genetic data, interpretation of the data, and drafting/revising of the manuscript. Yoshiko Murakami: study concept and design, analysis of the biological data, interpretation of the data, and drafting/revising of the manuscript. Kenjiro Kikuchi, Shuei Watanabe, Mizue Iai, Kazushi Miya, Ryuki Matsuura, and Rumiko Takayama: analysis of the clinical data and sample collection. Chihiro Ohba, Mitsuko Nakashima, Yoshinori Tsurusaki, and Noriko Miyake: analysis of the genetic data. Shin-ichiro Hamano and Hitoshi Osaka: analysis of the clinical data and sample collection. Kiyoshi Hayasaka: analysis of the clinical data and revising of the manuscript. Taroh Kinoshita: analysis of the biological data, interpretation of the data, and drafting/revising of the manuscript. Naomichi Matsumoto: study concept and design, analysis of the genetic data, interpretation of the data, and drafting/revising of the manuscript.

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REFERENCES

1. Kato M, Saitoh S, Kamei A, et al. A longer polyalanine expansion mutation in the *ARX* gene causes early infantile epileptic encephalopathy with suppression-burst pattern (Ohtahara syndrome). Am J Hum Genet 2007;81:361–366.
2. Saitsu H, Kato M, Mizuguchi T, et al. De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. Nat Genet 2008;40: 782–788.
3. Saitsu H, Kato M, Osaka H, et al. *CASK* aberrations in male patients with Ohtahara syndrome and cerebellar hypoplasia. Epilepsia 2012;53:1441–1449.
4. Kato M, Yamagata T, Kubota M, et al. Clinical spectrum of early onset epileptic encephalopathies caused by *KCNQ2* mutation. Epilepsia 2013;54:1282–1287.
5. Nakamura K, Kato M, Osaka H, et al. Clinical spectrum of *SCN2A* mutations expanding to Ohtahara syndrome. Neurology 2013;81:992–998.
6. Kato M. A new paradigm for West syndrome based on molecular and cell biology. Epilepsy Res 2006;70(suppl 1): S87–S95.
7. Saitsu H, Tohyama J, Kumada T, et al. Dominant-negative mutations in alpha-II spectrin cause West syndrome with severe cerebral hypomyelination, spastic quadriplegia, and developmental delay. Am J Hum Genet 2010;86:881–891.
8. Kurian MA, Meyer E, Vassallo G, et al. Phospholipase C beta 1 deficiency is associated with early-onset epileptic encephalopathy. Brain 2010;133:2964–2970.
9. Almeida AM, Murakami Y, Layton DM, et al. Hypomorphic promoter mutation in *PIGM* causes inherited glycosylphosphatidylinositol deficiency. Nat Med 2006;12: 846–851.
10. 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.
11. Johnston JJ, Gropman AL, Sapp JC, et al. The phenotype of a germline mutation in *PIGA*: the gene somatically mutated in paroxysmal nocturnal hemoglobinuria. Am J Hum Genet 2012;90:295–300.
12. 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.
13. Krawitz PM, Schweiger MR, Rodelsperger C, et al. Identity-by-descent filtering of exome sequence data identifies *PIGV* mutations in hyperphosphatasia mental retardation syndrome. Nat Genet 2010;42:827–829.
14. Kvarnung M, Nilsson D, Lindstrand A, et al. A novel intellectual disability syndrome caused by GPI anchor deficiency due to homozygous mutations in *PIGT*. J Med Genet 2013;50:521–528.
15. Maydan G, Noyman I, Har-Zahav A, et al. Multiple congenital anomalies-hypotonia-seizures syndrome is caused by a mutation in *PIGN*. J Med Genet 2011;48: 383–389.
16. Ng BG, Hackmann K, Jones MA, et al. Mutations in the glycosylphosphatidylinositol gene *PIGL* cause CHIME syndrome. Am J Hum Genet 2012;90:685–688.
17. DePristo MA, Banks E, Poplin R, et al. A framework for variation discovery and genotyping using next-generation DNA sequencing data. Nat Genet 2011; 43:491–498.
18. 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.
19. Wang K, Li M, Hakonarson H. ANNOVAR: functional annotation of genetic variants from high-throughput sequencing data. Nucleic Acids Res 2010;38:e164.
20. Molinari F, Raas-Rothschild A, Rio M, et al. Impaired mitochondrial glutamate transport in autosomal recessive neonatal myoclonic epilepsy. Am J Hum Genet 2005;76: 334–339.
21. Nozaki M, Ohishi K, Yamada N, Kinoshita T, Nagy A, Takeda J. Developmental abnormalities of glycosylphosphatidylinositol-anchor-deficient embryos revealed by Cre/loxP system. Lab Invest 1999;79:293–299.
22. Watanabe S, Murayama A, Haginoya K, et al. Schinzel-Giedion syndrome: a further cause of early myoclonic encephalopathy and vacuolating myelinopathy. Brain Dev 2012;34:151–155.
23. Barone R, Aiello C, Race V, et al. DPM2-CDG: a muscular dystrophy-dystroglycanopathy syndrome with severe epilepsy. Ann Neurol 2012;72:550–558.
24. Yang AC, Ng BG, Moore SA, et al. Congenital disorder of glycosylation due to *DPM1* mutations presenting with dystroglycanopathy-type congenital muscular dystrophy. Mol Genet Metab 2013;110:345–351.

25. Sener RN. Diffusion magnetic resonance imaging patterns in metabolic and toxic brain disorders. Acta Radiol 2004; 45:561–570.
26. Kuki I, Takahashi Y, Okazaki S, et al. Vitamin B6-responsive epilepsy due to inherited GPI deficiency. Neurology 2013; 81:1467–1469.
27. Aicardi J. Aicardi syndrome. Brain Dev 2005;27: 164–171.
28. Lehman AM, McFadden D, Pugash D, Sangha K, Gibson WT, Patel MS. Schinzel-Giedion syndrome: report of splenopancreatic fusion and proposed diagnostic criteria. Am J Med Genet A 2008;146A: 1299–1306.
29. Hoischen A, van Bon BW, Gilissen C, et al. De novo mutations of *SETBP1* cause Schinzel-Giedion syndrome. Nat Genet 2010;42:483–485.
30. Kranz C, Denecke J, Lehrman MA, et al. A mutation in the human *MPDU1* gene causes congenital disorder of glycosylation type If (CDG-If). J Clin Invest 2001;108:1613–1619.
31. Almeida AM, Murakami Y, Baker A, et al. Targeted therapy for inherited GPI deficiency. N Engl J Med 2007;356: 1641–1647.

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CASE REPORT

Sudden death in a case of megalencephaly capillary malformation associated with a de novo mutation in *AKT3*

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Abstract
Introduction Megalencephaly capillary malformation (MCAP) is a syndrome involving brain overgrowth, characterized by megalencephaly, capillary malformations, asymmetric growth, polymicrogyria, polydactyly, and syndactyly. Cerebellar tonsillar herniation (CTH) and ventriculomegaly are also observed in over half the patients with this syndrome. Early sudden death has been reported in MCAP, but its causes and the surgical strategies for its prevention remain unclear.
Case report Here, we report on a patient with MCAP who died suddenly at 5 months of age. He presented with progressive macrocephaly and hypotonia. MRI performed at 4 months of age showed tight posterior fossa, bilateral perisylvian polymicrogyria, enlargement of the straight sinus, and a thickened corpus callosum. However, since the patient did not exhibit capillary malformation, polydactyly, or syndactyly, a definitive diagnosis of MCAP could not be made. He died suddenly while asleep at home 1 month later. The sudden death of MCAP patients was previously attributed to CTH, convulsion, or arrhythmia. In this case, progressive cerebellar enlargement appeared to be the underlying cause. After the patient's death, using his preserved DNA, a missense mutation in the *AKT3* gene was identified. Vakt murine thymoma viral oncogene homologue (AKT) is a serine-threonine kinase that functions in the mammalian target of rapamycin (mTOR) pathway and plays an important role in cell proliferation.
Conclusion Accurate early diagnosis, including imaging and genetic analyses, and the recognition and treatment of critical conditions are required to prevent the sudden death of patients with MCAP.

Keywords Megalencephaly capillary malformation · Sudden death · *AKT3* mutation · Cerebellar tonsillar herniation · Posterior fossa decompression

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Introduction

Megalencephaly capillary malformation (MCAP), previously reported as macrocephaly-cutis marmorata telangiectatica congenita (M-CMTC) [1, 3, 9–11, 13, 14, 18, 19, 22, 24, 26, 27], is a rare overgrowth syndrome characterized by megalencephaly, capillary malformations, syndactyly, polymicrogyria, and connective tissue dysplasia. MCAP was first described in 1997 by Moore et al. and Clayton-Smith et al. [3, 19]. Since then, more than 140 cases have been

reported, primarily as genetic studies [17]. Megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH) is another megalencephaly syndrome with characteristics that partly overlap with those of MCAP. However, among patients with MPPH, the only consistent somatic feature is postaxial polydactyly [16].

Hydrocephalus and cerebellar tonsillar herniation (CTH) are frequently associated with MCAP, but neurosurgical approaches are not well reported, and the optimal surgical management strategies remain uncertain. The prognosis for MCAP varies from mild developmental delays to death, and while sudden death has been reported in eleven cases, the reasons for this outcome are still unclear [1–3, 5, 7, 8, 11, 13, 21, 27].

A recent report showed that both MCAP and MPPH are associated with germline and post-zygotic somatic mutations in three genes that encode members of the phosphoinositide-3-kinase (PI3K)—Vakt murine thymoma viral oncogene homologue (AKT)—mammalian target of rapamycin (mTOR) pathway, namely *PIK3R2*, *PIK3CA*, and *AKT3* [23]. The PI3K-AKT-mTOR pathway regulates cell growth, proliferation, metabolism, survival, and apoptosis, as well as angiogenesis, tumorigenesis, and brain development. The identification of the genetic anomalies associated with these syndromes will greatly contribute to our understanding of their pathophysiological mechanisms.

In the present report, we describe a case of MCAP in which the patient was found to have an *AKT3* mutation. He presented with progressive cerebellar enlargement and underwent sudden death before recognition of this serious condition.

Case presentation

History The patient was born after 39 weeks of gestation by vacuum extraction due to prolonged deceleration. He was admitted to the neonatal intensive care unit because of hypotonia and low Apgar scores, which were 4 at 1 min and 7 at 5 min. His birth weight was 3464 g (+1.2 SD above the mean), birth height was 58 cm (+4.3 SD), and head circumference (HC) was 38.5 cm (+3.7 SD). A thoracic echo-Doppler ultrasound showed a small patent foramen ovale at birth, and brain MR imaging performed 5 days later showed polymicrogyria around the bilateral perisylvian fissures and the cavum vergae (Fig. 1). The child was referred to our department at 3 months of age because of a significant increase in the HC, to four SDs above the mean (Fig. 2). He was still hypotonic and had inspiratory stridor. We suspected that he suffered from MCAP or MPPH with bilateral perisylvian polymicrogyria based on the MRI findings at birth and a capillary malformation in the midline of his face, which was prominent only when he was crying, even though he exhibited no other clinical features such as syndactyly or polydactyly. However, we could not render a definitive diagnosis of MCAP at that time. A second MR image at 4 months of age revealed enlargement of the cerebellum and tight posterior fossa, without ventriculomegaly (Fig. 3). Just 1 month after the MRI, the patient died suddenly while sleeping at home. Autopsy CT imaging did not show any abnormal findings, such as subdural hematoma.

Genetic diagnosis We obtained a blood sample and extracted genomic DNA for genetic analysis 1 month before the sudden death. The sample was analyzed for PI3K-AKT-mTOR pathway mutations by targeted sequencing using the SureSelect XT Target Enrichment System for Illumina Paired-End Sequencing Library kit (Agilent Technologies, Santa Clara, CA) and the Illumina HiSeq 2000 sequencer (San Diego, CA). A heterozygous missense mutation in the *AKT3* gene (exon7, c.686A>A/G, p.Asn229Ser) was found. Sanger sequencing confirmed the presence of the mutation in this patient and the absence of the mutation in his parents, thereby indicating that the mutation occurred de novo. Genetic examination of the patient's healthy older sister was not performed (Fig. 4).

Discussion

Diagnostic criteria for MCAP and related disorders

Megalencephaly capillary malformation (MCAP) is characterized by prenatal overgrowth, somatic and cerebral asymmetry, megalencephaly, developmental delay, connective tissue abnormalities, capillary malformation, and digital abnormalities including syndactyly or polydactyly. This syndrome was also known as macrocephaly-cutis marmorata telangiectatica congenital (M-CMTC), but Toriello et al. proposed that this condition be renamed MCAP because the vascular anomalies associated with this syndrome were capillary malformations [25]. Wright et al. reviewed the skin lesions of previously reported MCAP cases [26] and agreed that the vascular anomalies were not true cutis marmorata telangiectatica congenital, but rather capillary malformations in the form of reticulated portwine stains. Neuroimaging studies of patients with MCAP have indicated the presence of brain asymmetry, polymicrogyria, ventriculomegaly, white matter abnormalities, CTH, cavum septum pellucidum or cavum vergae, enlarged or dilated venous sinuses, and a thickened corpus callosum [5, 13]. CTH and ventriculomegaly are observed in more than half of the patients and are considered distinctive features of this syndrome [5].

Various sets of diagnostic criteria for MCAP were proposed by Franceschini et al. and Robertson et al. in 2000 and Wright et al. in 2009 [9, 24, 26]. In contrast, megalencephaly-polymicrogyria-polydactyly-hydrocephalus syndrome (MPPH) was recognized as another megalencephaly syndrome,

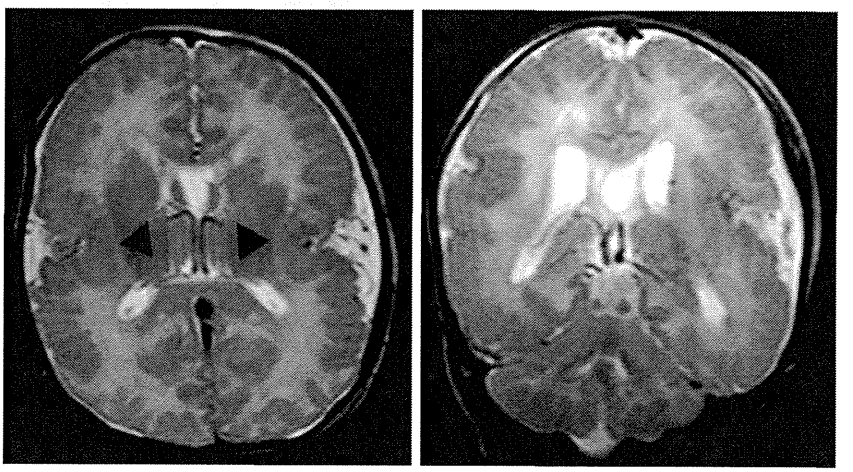

Fig. 1 T2-weighted brain MR images at 5 days of age. *Left*: axial image, showing bilateral perisylvian polymicrogyria (*arrowheads*) without ventriculomegaly. *Right*: coronal image

clinically distinct from MCAP, based on its somatic features [16]. In 2009, Grip et al. hypothesized that MPPH and MCAP represent either one single or two closely related disorders and that they are caused by mutations in the same gene or different genes in the same functional pathway [12]. Recently, mutations in genes encoding PI3K-AKT-mTOR pathway members have

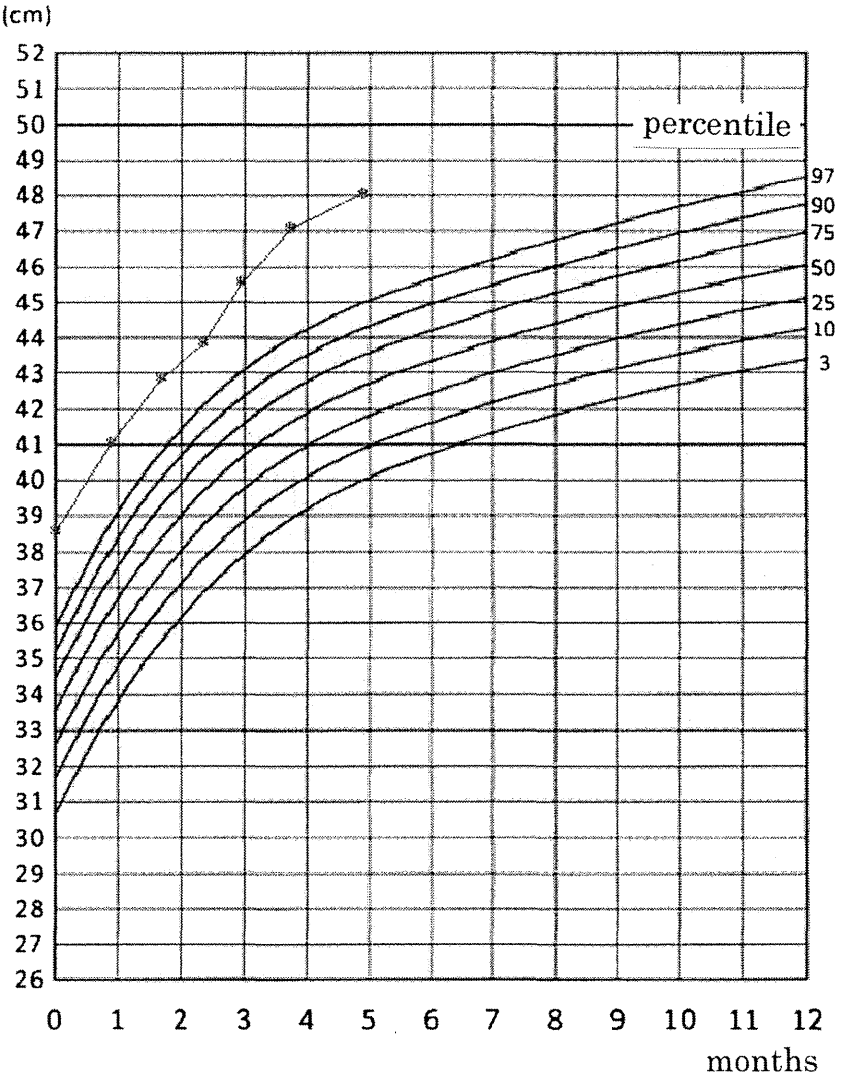

Fig. 2 Growth chart demonstrating rapid deviation of head circumference from the percentile lines normally obtained soon after birth

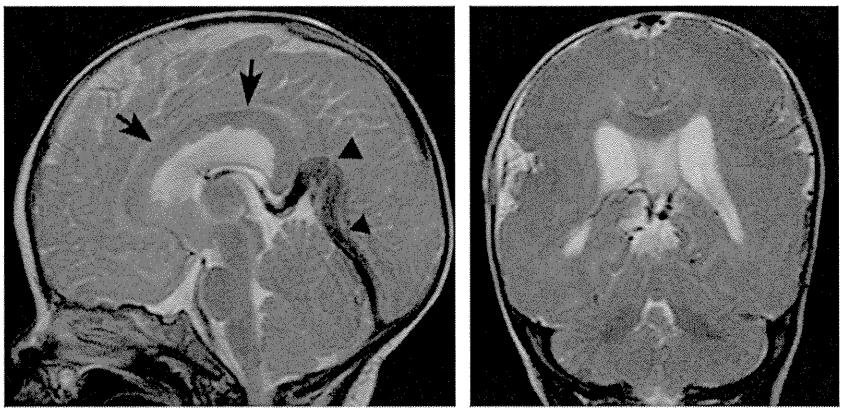

Fig. 3 T2-weighted brain MR images at 4 months of age. *Left*: Sagittal image, showing tight posterior fossa with enlargement of the straight sinus (*arrowheads*) and the corpus callosum (*arrows*) *Right*: Coronal image, showing progressive enlargement of the cerebellum compared with Fig. 1

been identified in patients with MCAP and MPPH [23]. Mutations in *PIK3R2* were identified in MPPH, while mutations in *PIK3CA* were identified in MCAP. *AKT3* mutations were identified in patients with atypical MPPH features that overlapped both disorders. Mutations in these three genes have now been identified in 39 cases of MCAP and MPPH [17, 20, 23]. Like the case presented here, some patients are missing certain typical features of these diseases; therefore, genetic analysis is required for definitive diagnoses of these related disorders.

Etiology of MCAP and MPPH

The identification of the genetic anomalies in patients diagnosed with MCAP or the related MPPH will facilitate our understanding of the pathophysiology of these syndromes. The identified mutations were found to result in gain-of-function and activation of the PI3K-AKT-mTOR pathway, which regulates cell growth and proliferation. In our patient, an *AKT3* mutation was identified. AKT, also known as protein kinase B, is an important signaling molecule within this pathway. The finding that these anomalies directly impact the PI3K-AKT-mTOR signaling pathway is consistent with the overgrowth pathologies associated with these syndromes [23]. CTH and ventriculomegaly are present in 64 and 81 % of MCAP cases, respectively [15]. Progressive overgrowth of the cerebellum leads to CTH, while cerebral overgrowth leads to megalencephaly. Hydrocephalus could be a secondary event resulting from the reduced drainage of cerebrospinal fluid following the development of CTH or megalencephaly, although the mechanism underlying such a development is unclear. Conway et al. suggested that the enlarged cerebellum obstructs the venous outflow, causing reduced cerebrospinal fluid absorption [4]. They also suggested a second possible mechanism involving compression of the cerebral aqueduct due to the increased crowding of the posterior fossa contents [4].

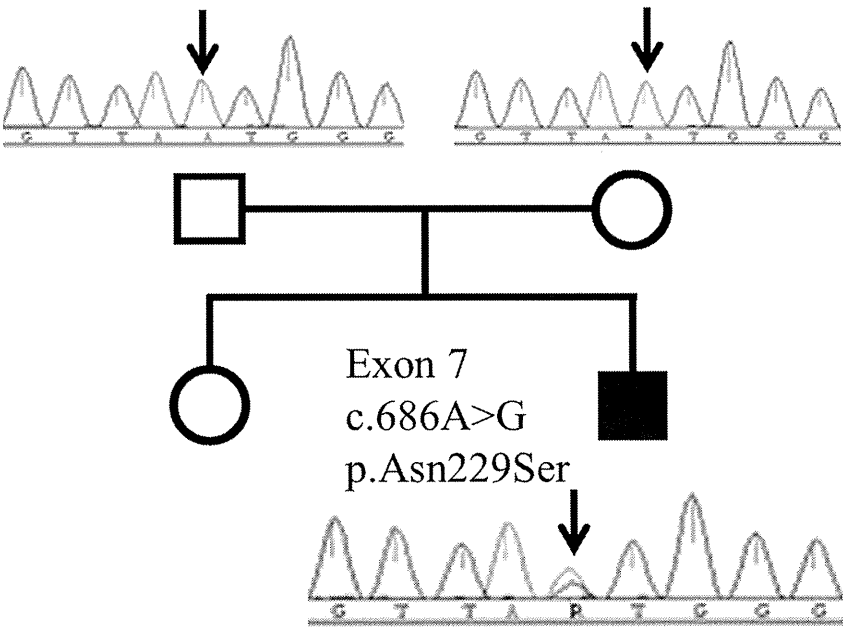

Fig. 4 Pedigree and *AKT3* mutation analysis

Surgical treatments for MCAP

An estimated 50 % of MCAP cases may benefit from neurosurgical procedures, including ventriculo-peritoneal shunt (VPS) or posterior fossa decompression [15]. However, MCAP cases are rarely reported in the neurosurgical literature [4, 14, 18, 22], and a defined surgical strategy based on the etiology of this syndrome has not yet been established. Of the few reported cases, Mitha et al. successfully treated two patients with ventriculomegaly, regarded as obstructive hydrocephalus, with an endoscopic third ventriculostomy [18]. In addition, Martinez-Lage et al. reported that use of VPS to treat the hydrocephalus in MCAP patients might prevent CTH development or stop its progression [14]. Although the most beneficial treatment for hydrocephalus or CTH is still under discussion, surgical intervention can help relieve symptoms and may be life-saving, because as discussed below, hydrocephalus and CTH may be related to the occurrence of sudden death in this syndrome.

Sudden death in patients with MCAP

Eleven cases of early sudden death have been reported in infants and young children diagnosed with MCAP in the absence of genetic analysis (Table 1) [1–3, 5, 7, 8, 11, 13, 21, 27]. The cause of death was unknown in seven cases, while convulsions and respiratory distress occurred in two cases each. The reporting authors suggested that the deaths could be attributed to congenital cardiac malformations, arrhythmia, CTH, or sinus thrombosis. A survey of these cases indicated that they can be classified into two groups based on the patient's age at death. The early death group included four patients who died during the neonatal period, and the late death group included seven patients who died between 5 and 33 months of age. In the early death group, none of the patients exhibited CTH, while in the late death group, four of the seven patients presented with CTH. This finding suggests that CTH is an acquired condition, consistent with previous reports [4, 12]. All seven of the patients in the late death group showed evidence of ventriculomegaly and/or CTH, and four of them underwent VPS. Although four of these patients had associated arrhythmias or congenital cardiac anomalies, the other three had no other associated conditions. We suspect that the uncontrolled hydrocephalus and/or CTH in these cases was causally related to the occurrence of sudden death. Our patient belonged to the late death group and had progressive overgrowth of the cerebellum without any history of cardiac symptoms or epilepsy. Therefore, we think that tight posterior fossa may have led to his sudden death. The results of autopsy CT imaging showed no new lesions.

Dhamija et al. recently reported that nocturnal polysomnography (nPSG) is useful for assessing the nocturnal apnea and the surgical decisions for children with Chiari type 1 malformation [6]. They found that both the central apnea and cortical arousal indexes were correlated with the degree of CTH and the subarachnoid space effacement. Thus, nPSG may be a useful prognostic tool in predicting the risk of sudden death in MCAP patients with CTH.

Franklin et al. reported that both unilateral hypertrophy of the vocal cords and CTH may lead to airway obstruction in MCAP patients [10]. The patient in our study also had inspiratory stridor, which may have been related to airway

Table 1 Eleven cases reported of early sudden death in infants and young children diagnosed with MCAP in the absence of genetic analysis

No.	Authors	Age at death	Cause of death	CTH	VM	Operation	Comments
1	Clayton-Smith, 1997	2 days	Respiratory distress	−	+	−	Sagittal sinus thrombosis, bilateral angiomatosis at the apices of both lungs
2	Akcar, 2004	17 days	Unknown	−	+	−	Giant atrial aneurysm, atrial septal defect
3	Lapunzina, 2004	1 month	Unknown	−	−	−	Generalized edema
4	Giuliano 2004	Neonatal period	Unknown	−	−	−	Complex congenital heart disease, arrhythmia
5	Duenas-Arias, 2009	5 months	Unknown	−	+	VPS	TOF, death 15 days after VPS
6	Ecran, 2013	6 months	Unknown (during sleep)	+	−	−	Sagittal sinus thrombosis, TOF, atrial septal aneurysm
7	Barnicoat, 1996	6 months	Respiratory distress	−	+	VPS	Death 1 month after shunt revision
8	Reardon, 1996	10 months	Convulsion	+	+	VPS	Reported as Proteus syndrome, autopsy
9	Yano, 2001	19 months	Unknown (during sleep)	−	+	−	Atrial flutter, right ventricular hypertrophy, atrioventricular block
10	Conway, 2007	30 months	Convulsion	+	+	VPS	Atrial flutter in newborn period
11	Yano, 2001	33 months	Unknown (during sleep)	+	+	VPS	

CTH cerebellar tonsillar herniation, *TOF* tetralogy of fallot, *VM* ventriculomegaly, *VPS* ventriculoperitoneal shunt, *Case 1-4* early death group, *Case 5-11* late death group

obstruction due to laryngeal hypertrophy, although we did not examine the larynges.

In summary, we propose that the prevention of sudden death in MCAP patients requires a definitive diagnosis, evaluation of the deteriorating process, and optimal surgical intervention. A definitive diagnosis should be based on both clinical findings and genetic analysis. The follow-up should include careful radiological analysis, cardiological evaluation, and laryngeal examination. For patients exhibiting CTH, nPSG should be performed. With respect to surgical intervention, patients with CTH and diagnosed with central apnea by nPSG should undergo a posterior fossa decompression. For patients with CTH and ventriculomegaly, VPS may be initially performed. Continued advances in the genetic analysis of MCAP patients will clarify the genotype-phenotype correlations and lead to an increased understanding of the pathophysiology of this disease and improved management strategies.

Conclusions

Here, we described a patient with MCAP who died suddenly at 5 months of age and was genetically diagnosed with a de novo *AKT3* mutation. Based on the analysis of this case and other sudden deaths in young children with MCAP, the cause of his death was probably tight posterior fossa. Early diagnosis and appropriate surgical decision-making can be difficult in cases in which the clinical symptoms are not definitive. In such cases, genetic diagnosis and serial neuroimaging analysis and appropriate management with neurosurgeons, neurologists, and cardiologists are required to improve the likelihood of a positive outcome.

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Conflict of interest The authors declare that they have no conflict of interest.

References

1. Akcar N, Adapinar B, Dinleyici C, Durak B, Özkan IR (2004) A case of macrocephaly-cutis marmorata telangiectatica congenita and review of neuroradiologic features. Ann Genet 47:261–265
2. Barnicoat A, Salman M, Chitty L, Baraster M (1996) A distinctive overgrowth syndrome with polysyndactly. Clin Dysmorphol 5:339–346
3. Clayton-Smith J, Kerr B, Brunner H, Tranebjaerg L, Magee A, Hennekam RC, Mueller RF, Brueton L, Super M, Steen-Johnsen J, Donnai D (1997) Macrocephaly with cutis marmorata, haemangioma and syndactyly—a distinctive overgrowth syndrome. Clin Dysmorphol 6:291–302
4. Conway RL, Danielpour M, Graham JM Jr (2007) Surgical management of cerebellar tonsillar herniation in three patients with macrocephaly-cutis marmorata telangiectatica congenita. J Neurosurg 106(4 Suppl):296–301
5. Conway RL, Pressman BD, Dobyns WB, Danielpour M, Lee J, Sanchez-Lara PA, Bulter MG, Zackai E, Campbell L, Saitta SC, Clericuzio CL, Milunsky JM, Hoyme HE, Shieh J, Moeschler JB, Crandall B, Lauzon JL, Viskochil DH, Harding B, Graham JM Jr (2007) Neuroimaging findings in macrocephaly-capillary malformation: a longitudinal study of 17 patients. Am J Med Genet A 143: 2981–3008
6. Dhamija R, Wetjen NM, Slocumb NL, Mandrekar J, Kotagal S (2013) The role of nocturnal polysonography in assessing children with Chiari type 1 malformation. Clin Neurol Neurosurg 115:1837–1841
7. Dueñas-Arias JE, Arámbula-Meraz E, Frías-Castro LO, Ramos-Payán R, Quibrera-Matienzo JA, Luque-Ortega F, Aguilar-Medina EM (2009) Tetralogy of Fallot associated with macrocephaly-capillary malformation syndrome: a case report and review of the literature. J Med Case Rep 3:9215
8. Ecran TE, Oztun F, Celkan T, Bor M, Kizilkilic O, Vural M, Perk Y, Islak C, Tuysuz B (2013) Macrocephaly-capillary malformation syndrome in a newborn with tetralogy of Fallot and sagittal sinus thrombosis. J Child Neurol 28:115–119
9. Franceschini P, Licata D, Di Cara G, Guala A, Franceschini D, Genitori L (2000) Macrocephaly-cutis marmorata telangiectatica congenita without cutis marmorata? Am J Med Genet 90:265–269
10. Franklin B, Gasco J, Rangel-Castilla L, Nauta HJ (2009) Apnea and macrocephaly-cutis marmorata telangiectatica congenita. Brain Dev 31:706–709
11. Giuliano F, David A, Edery P, Sigaudy S, Bonneau D, Cormier-Daire V, Philip N (2004) Macrocephaly-cutis marmorata telangiectatica congenita: seven cases including two with unusual cerebral manifestations. Am J Med Genet 126A:99–103
12. Gripp KW, Hopkins E, Vinkler C, Lev D, Malinger G, Lerman-Sagie T, Dobyns WB (2009) Significant overlap and possible identity of macrocephaly capillary malformation and megalencephaly polymicrogyria-polydactyly hydrocephalus syndromes. Am J Med Genet Part A 149A:868–876
13. Lapunzina P, Gairi A, Delicado A, Mori MA, Torres ML, Goma A, Navia M, Pajares IL (2004) Macrocephaly-cutis marmorata telangiectatica congenita: report of six new patients and a review. Am J Med Genet 130A:45–51
14. Martínez-Lage JF, Guillén-Navarro E, Almgro MJ, Felipe-Murcia M, Lopez-Guerrero AL, Galarza M (2010) Hydrocephalus and Chiari type 1 malformation in macrocephaly-cutis marmorata telangiectatia congenita: a case-based update. Childs Nerv Syst 26:13–18
15. Mirzaa GM, Conway RL, Gripp KW, Lerman-Sagie T, Siegel DH, deVries LS, Lev D, Kramer N, Hopkins E, Graham JM Jr, Dobyns WB (2012) Megalencephaly-capillary malformation (MCAP) and megalencephaly–polydactyly–polymicrogyria–hydrocephalus (MPPH) syndromes: two closely related disorders of brain overgrowth and abnormal brain and body morphogenesis. Am J Med Genet Part A158A:269–291
16. Mirzaa GM, Dodge NN, Glass I, Day C, Gripp K, Nicholson L, Straub V, Voit T, Dobyns WB (2004) Megalencephaly- and perisylvian polymicrogyria with postaxial polydactyly and hydrocephalus: a rare brain malformation syndrome associated with mental retardation and seizures. Neuropediatrics 35:353–359

17. Mirzaa GM, Rivière JB, Dobyns WB (2013) Megalencephaly syndromes and activating mutations in the PI3K-AKT pathway: MPPH and MCAP. Am J Med Genet Part C 163C:122–130
18. Mitha AP, Bullivant KJ, Lauzon JL, Hader WJ (2009) Endoscopic third ventriculostomy to treat hydrocephalus associated with macrocephaly-cutis marmorata telangiectatica congenita. J Neurosurg Pediatr 4:397–401
19. Moore CA, Toriello HV, Abuelo DN, Bull MJ, Curry CJR, Hall BD, Higgins JV, Stevens CA, Twersky S, Weksberg R, Dobyns WB (1997) Macrocephaly-cutis marmorata telangiectatica congenita: a distinct disorder with developmental delay and connective tissue abnormalities. Am J Med Genet 70:67–73
20. Nakamura K, Kato M, Tohyama J, Shiohama T, Hayasaka K, Nishiyama K, Kodera H, Nakashima M, Tsurusaki Y, Miyake N, Matsumoto N, Saitsu H (2014) AKT3 and PIK3R2 mutations in two patients with megalencephaly-related syndromes: MCAP and MPPH. Clin Genet 85:396–398
21. Reardon W, Harding B, Winter RM, Baraitser M (1996) Hemihypertrophy, hemimegalencephaly, and polydactyly. Am J Med Genet 66:144–149
22. Rekate H (2007) Macrocephaly-cutis marmorata telangiectatica congenita. J Neurosurg 106(4 Suppl):292–293
23. Rivière J-B, Mirzaa GM, O'Roak BJ, Beddaoui M, Alcantara D, Conway RL, St-Onge J, Schwartzentruber JA, Gripp KW, Nikkel SM, Worthylake T, Sullivan CT, Ward TR, Butler HE, Kramer NA, Albrecht B, Armour CM, Armstrong L, Caluseriu O, Cytrynbaum C, Drolet BA, Innes AM, Lauzon JL, Lin AE, Mancini GMS, Meschino WS, Reggin JD, Saggar AK, Lerman-Sagie T, Uyanik G, Weksberg R, Zirn B, Beaulieu CL, Canada Consortium FORGE, Majewski J, Bulman DE, O'Driscoll M, Shendure J, Graham JM Jr, Boycott KM, Dobyns WB (2012) De novo germline and postzygotic mutations in AKT3, PIK3R2 and PIK3CA cause a spectrum of related megalencephaly syndromes. Nat Genet 44:934–940
24. Robertson SP, Gattas M, Rogers M, Ades LC (2000) Macrocephaly cutis marmorata telangiectatica congenita: report of five patients and a review of the literature. Clin Dysmorphol 9:1–9
25. Toriello HV, Mulliken JB (2007) Accurately renaming macrocephaly-cutis marmorata telangiectatica congenital (M-CMTC) as macrocephaly-capillary malformation (M-CM). Am J Med Genet Part A 143A:2009
26. Wright DR, Frieden IJ, Orlow SJ, Shin HT, Chamlin S, Schaffer JV, Paller AS (2009) The misnomer "macrocephaly-cutis marmorata telangiectatica congenita syndrome" report of 12 new cases and support for revising the name to macrocephaly-capillary malformations. Arch Dermatol 145:287–293
27. Yano S, Watanabe Y (2001) Association of arrhythmia and sudden death in macrocephaly-cutis marmorata telangiectatica congenita syndrome. Am J Med Genet 102:149–152

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SHORT COMMUNICATION

A girl with West syndrome and autistic features harboring a *de novo TBL1XR1* mutation

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Recently, *de novo* mutations in *TBL1XR1* were found in two patients with autism spectrum disorders. Here, we report on a Japanese girl presenting with West syndrome, Rett syndrome-like and autistic features. Her initial development was normal until she developed a series of spasms at 5 months of age. Electroencephalogram at 7 months showed a pattern of hypsarrhythmia, which led to a diagnosis of West syndrome. Stereotypic hand movements appeared at 8 months of age, and autistic features such as deficits in communication, hyperactivity and excitability were observed later, at 4 years and 9 months. Whole exome sequencing of the patient and her parents revealed a *de novo TBL1XR1* mutation [c.209 G>A (p.Gly70Asp)] occurring at an evolutionarily conserved amino acid in an F-box-like domain. Our report expands the clinical spectrum of *TBL1XR1* mutations to West syndrome with Rett-like features, together with autistic features.
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TBL1XR1 at 3q26.32 encodes transducin β-like 1 X-linked receptor 1, a co-repressor of nuclear hormone transcription factors that is required for β-catenin–Tcf-mediated Wnt signaling.[1–3] Recently, two *de novo TBL1XR1* mutations were found in 2 of 2446 patients with autism spectrum disorders, suggesting an association between *TBL1XR1* mutations and autism.[4,5] Here, we present a third case with a *de novo TBL1XR1* mutation, showing West syndrome, Rett-like and autistic features.

CASE REPORT

This report concerns a 5-year-old girl who is the offspring of unrelated healthy Japanese parents. She was born at 39 weeks of gestation without asphyxia after an uneventful pregnancy. Her birth weight, birth length and head circumference were 3088 g (+0.3 standard deviation (s.d.)), 51.1 cm (+1.0 s.d.) and 34.0 cm (+0.5 s.d.), respectively. She showed social smiling and head control at 3 and 4 months of age, respectively. Then at 5 months she developed a series of spasms occurring 5–6 times a day, when her head control became unstable. Electroencephalography at 7 months of age showed hypsarrhythmia patterns (Figure 1a), which led to a diagnosis of West syndrome. Brain magnetic resonance imaging showed no structural brain anomalies (Figures 1a and c). Administration of adrenocorticotropic hormone therapy only temporarily reduced the frequency of spasms.

On examination at 7 months of age, the patient's weight, height and head circumference were 8720 g (+1.2 s.d.), 69.5 cm (+0.9 s.d.) and 42 cm (−0.6 s.d.), respectively. Mild dysmorphic features were observed, including a long palpebral fissure, thick eyebrows and downturned corners of the mouth. The patient showed no eye fixation and pursuit, as well as no social smile. Her muscle tone was mildly hypotonic. Despite being given extensive treatments including adrenocorticotropic hormone therapy in combination with valproic acid, nitrazepam, vitamin B6, topiramate, clonazepam and clobazam, she continued to exhibit frequent subtle tonic seizures with eyelid opening. At 8 months of age, stereotypical hand movements appeared that resembled hand-washing.

Laboratory examination revealed elevated serum levels of several components: lactic acid (39.9 mg dl^{-1}, compared with a normal range of 5.0–20.0 mg dl^{-1}), pyruvate (2.79 mg dl^{-1}, normal range 0.3–0.9 mg dl^{-1}) and alanine (1447 nmol ml^{-1}, normal range 180–470 nmol ml^{-1}). However, following vitamin B1 treatment, both pyruvate and alanine serum levels returned to normal. The levels of lactic acid and pyruvate in cerebrospinal fluid appeared normal (14.6 and 0.65 mg dl^{-1}, respectively). Respiratory-chain enzymes, using muscle homogenates and fibroblasts, and mitochondrial DNA sequence analysis were all normal, and pathological examination of muscle specimens revealed no specific findings. Repeated examination of both serum lactic acid and pyruvate levels also showed no abnormalities.

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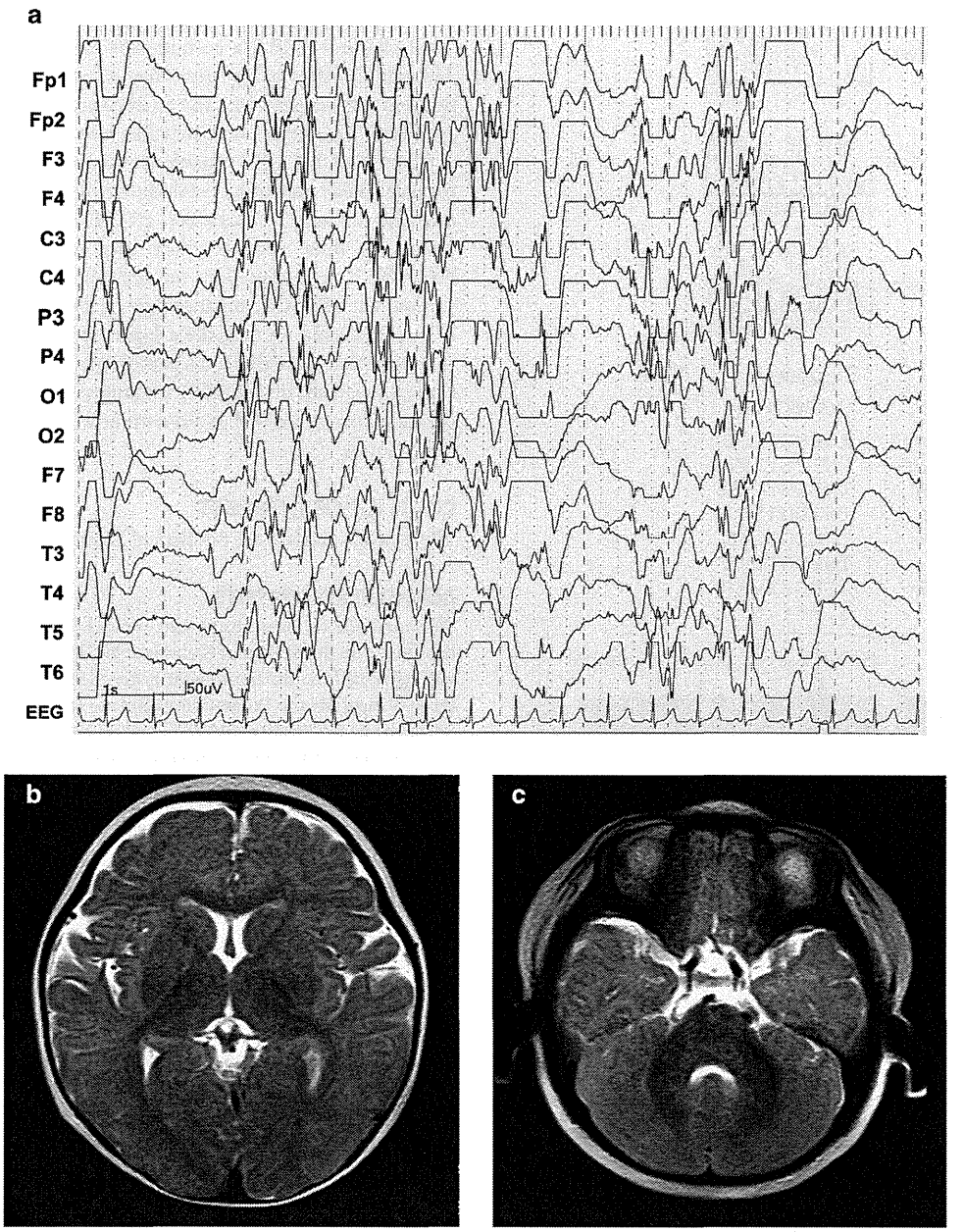

Figure 1 Electroencephalogram (EEG) and brain magnetic resonance imaging in the patient at 7 months of age. (**a**) Interictal EEG showed high-amplitude multifocal spikes with irregular slow waves consistent with a finding of hypsarrhythmia. T2-weighted axial images through the basal ganglia (**b**) and the cerebellum (**c**) showed no abnormalities.

After contracting a fever at the age of 1 year and 10 months, her seizures were controlled using a combination therapy of topiramate, valproic acid, clobazam and vitamin B1. However, the patient still could not speak any meaningful words at 4 years and 9 months of age but could walk with support; she had a developmental quotient of 13. At this age she still showed stereotypic hand movements as well as autistic features such as deficits in communication, hyperactivity and excitability.

RESULTS AND DISCUSSION

G-banded karyotyping was normal (46,XX). No pathological copy number aberrations were detected by the 2.7M Array (Affymetrix, Santa Clara, CA, USA). Whole exome sequencing of the patient and her parents was performed. Genomic DNA of blood leukocytes was captured using the SeqCap EZ Exome Library v2.0 (Roche NimbleGen, Madison, WI, USA), and sequenced with on HiSeq2000 (Illumina, San Diego, CA, USA). Variants were detected as previously described.[6] Variants with a Phred-like consensus quality score of >100 were considered as candidate variants. We found a total of four *de novo* candidate variants, in which two mutations were further validated as *de novo* by Sanger sequencing: *SELPLG* NM_001206609.1: c.794C>T (p.Thr265Met) and *TBL1XR1* NM_024665.4:c.209G>A (p.Gly70Asp). The other two variants were transmitted from her mother, demonstrating that the two variants were falsely uncalled in the mother. Neither of the two *de novo* mutations was found in the 6500 National Heart, Lung, and Blood Institute exomes nor in our