rehabilitation, he had full-range visual pursuit, a social smile, and incomplete head control. Although his spasticity improved, exaggerated deep tendon reflexes with synergic voluntary movement of the distal part of the extremities were recognized. An EEG at 1 year of age showed no epileptic discharges. His present developmental quotient is below 20. He did not show hematuria, muscular cramps, intracranial aneurysms, or cataracts. His elder sister was found to have an intraventricular hemorrhage two days after birth and underwent a V-P shunt. Her development was almost normal, and internal strabismus was noted. Unfortunately, she died in an accident at the age of four, and so her DNA was unavailable (Figure 1B).

Genomic DNA was isolated from peripheral blood leukocytes according to standard methods. DNA for mutation screening was amplified by illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare, Buckinghamshire, UK). The DNA of family members of individual 1 was isolated from saliva samples with Oragene (DNA Genotek Inc., Ontario, Canada). Exons 2 to 48 covering the entire *COL4A2* coding region (GenBank accession number NM_001846.2) were examined by high-resolution melting curve (HRM) analysis or directly sequenced (for exon 46). The samples showing an aberrant melting curve pattern in the HRM analysis were sequenced. PCR primers and conditions are shown in Table S1, available online. All the mutations were verified with genomic DNA as a template. Two heterozygous mutations, c.3455G>A (p.Gly1152Asp) in individual 1 and c.3110G>A (p.Gly1037Glu) in individual 2, were identified. Both mutations occur at evolutionary conserved Gly residues in the Gly-X-Y repeats (Figure 1D), suggesting that the two mutations may alter the collagen IV $\alpha1\alpha1\alpha2$ heterotrimers. These mutations were absent in 200 normal Japanese controls, and our evaluation with web-based prediction tools strongly suggested that these substitutions are pathogenic (Table S2). Screening for *COL4A1* mutations was negative for both individuals (data not shown). The c.3455G>A mutation was found in the proband's mother and the maternal uncle, who showed very mild monoparesis of the left upper extremity and congenital left hemiplegia, respectively, and in maternal grandfather who is asymptomatic (Figures 1A and 1B). Therefore the c.3455G>A mutation can be considered as a pathogenic mutation with incomplete penetrance. The c.3110G>A mutation in individual 2 was not found in his parents, indicating that this mutation occurred de novo (Figure 1C).

Here we report two individuals with porencephaly who harbor *COL4A2* mutations. In individual 2, the mutation occurred de novo. It is noteworthy that individual 2's elder sister also suffered from an intraventricular hemorrhage. A coincidental phenocopy in the sister is possible and would be consistent with de novo occurrence of the mutation. Alternatively, the sister might have the same mutation, which could be inherited from either one of the parents with a germline-mosaic mutation, though it was impossible to examine the sister because her sample is unavailable. Thus, with the present data, we concluded that the c.3110G>A mutation occurred de novo. On the other hand, the mutation in individual 1 was inherited from his mildly affected mother. In addition, congenital hemiplegia is observed in familial members of individual 1; the segregation of the c.3455G>A mutation is consistent with a dominant trait with incomplete penetrance. Such incomplete penetrance also has been reported in familial porencephalies with *COL4A1* mutations,[8,9] suggesting that abnormalities of collagen IV $\alpha1\alpha1\alpha2$ heterotrimers may conspire with other risk factors. The porencephalic cyst was unilateral in individual 1 and bilateral in individual 2, who required shunting, indicating variable severities caused by the different *COL4A2* mutations. Most porencephalic cysts caused by *COL4A1* mutations are unilateral;[9] however, Meuwissen et al. recently reported *de novo COL4A1* mutations in sporadic extensive bilateral porencephaly resembling hydranencephaly, indicating similar variable severities caused by *COL4A1* mutations.[10] Thus the involvement of *COL4A1* and *COL4A2* abnormalities should be considered in porencephaly and related pre- and perinatal cerebral hemorrhages, regardless of their severities.

It has been reported that *COL4A1* mutations cause a variety of phenotypes, including porencephaly, infantile hemiplegia, and cerebral small vessel diseases involving both ischemic stroke and intracerebral hemorrhage with radiological features of lacunar infarction, and leukoaraiosis in adult individuals.[9,15–18] The phenotypes in the central nervous system are often accompanied by ocular features (cataracts, retinal vessel tortuosity and hemorrhage, and defects of the anterior segment of the eye), nephropathy, and muscle cramps.[9,16,17] Considering the common pathological mechanism between *COL4A1* and *COL4A2* mutations (abnormalities of collagen IV $\alpha1\alpha1\alpha2$ heterotrimers), *COL4A2* mutations also may be involved in small vessel diseases that can be manifested in adulthood. Supporting this idea, mice lines harboring *Col4A2* point mutations showed cataracts, abnormalities of the lens and the cornea, and cerebral abnormalities.[14] Thus it is important to identify mutations in both *COL4A1* and *COL4A2* in individuals with porencephaly as well as in asymptomatic carriers, for whom the prevention of stroke and genetic counseling are quite important. Identification of pathogenic mutations in individuals with porencephaly is of great interest for obstetricians and pediatricians, and for neurologists working for adult individuals.

In summary, we have identified mutations in *COL4A2* as a genetic cause of both sporadic and familial porencephaly. Our data further support the importance of genetic testing in porencephaly and related pre- and perinatal cerebral hemorrhages for which the genetic predisposition is gradually being uncovered.

Supplemental Data

Supplemental Data include two tables and can be found with this article online at http://www.cell.com/AJHG/.

Acknowledgments

We would like to thank all the individuals and their families for their participation in this study. This work was supported by research grants from the Ministry of Health, Labour and Welfare (K.H., N. Miyake, H.O., M.K., N. Matsumoto, and H.S.), the Japan Science and Technology Agency (N. Matsumoto), the Strategic Research Program for Brain Sciences (N. Matsumoto), and a Grant-in-Aid for Scientific Research on Innovative Areas-(Foundation of Synapse and Neurocircuit Pathology)-from the Ministry of Education, Culture, Sports, Science and Technology of Japan (N. Matsumoto), a Grant-in-Aid for Scientific Research from Japan Society for the Promotion of Science (H.O., N. Matsumoto), a Grant-in-Aid for Young Scientist from Japan Society for the Promotion of Science (H.D., N. Miyake, H.S.) and a grant from the Takeda Science Foundation (N. Miyake and N. Matsumoto). This work has been done at the Advanced Medical Research Center, Yokohama City University, Japan.

Received: September 27, 2011
Revised: November 4, 2011
Accepted: November 17, 2011
Published online: December 29, 2011

Web Resources

The URLs for data presented herein are as follows:

Clustal W, http://www.genome.jp/tools/clustalw/
GenBank, http://www.ncbi.nlm.nih.gov/Genbank/
Online Mendelian Inheritance in Man (OMIM), http://www.omim.org

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nature genetics

Mutations affecting components of the SWI/SNF complex cause Coffin-Siris syndrome

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By exome sequencing, we found *de novo SMARCB1* mutations in two of five individuals with typical Coffin-Siris syndrome (CSS), a rare autosomal dominant anomaly syndrome. As *SMARCB1* encodes a subunit of the SWItch/Sucrose NonFermenting (SWI/SNF) complex, we screened 15 other genes encoding subunits of this complex in 23 individuals with CSS. Twenty affected individuals (87%) each had a germline mutation in one of six SWI/SNF subunit genes, including *SMARCB1*, *SMARCA4*, *SMARCA2*, *SMARCE1*, *ARID1A* and *ARID1B*.

Chromatin remodeling factors regulate the gene accessibility and expression by dynamic alteration of chromatin structure. SWI/SNF complexes have important roles in lineage specification, maintenance of stem cell pluripotency and tumorigenesis[1–5]. These complexes are composed of evolutionarily conserved core subunits and variant subunits. Brahma-associated factor (BAF) and Polybromo BAF (PBAF) complexes constitute two major subclasses[1–5]. It has been suggested that the BAF complex is similar to the yeast SWI/SNF complex and that the PBAF complex is more like the chromatin remodelling complex (RSC) in yeast, which is required for cell cycle progression through mitosis[6]. However, several subunits that are common to both BAF and PBAF complexes are predicted to be related to the regulation of lineage- and tissue-specific gene expression[2].

Coffin-Siris syndrome (MIM 135900) is a rare congenital anomaly syndrome characterized by growth deficiency, intellectual disability, microcephaly, coarse facial features and hypoplastic nail of the fifth finger and/or toe (**Fig. 1** and **Supplementary Table 1**)[7]. The majority of affected individuals represent sporadic cases, which is compatible with an autosomal dominant inheritance mechanism. The genetic cause for this syndrome has not been elucidated.

To identify the genetic basis of CSS, we performed whole-exome sequencing of five typical affected individuals (**Supplementary Methods**). Taking into account our model that assumes that an abnormality in a causal gene would be shared in two or more subjects, 51 variants were identified as candidates (**Supplementary Table 2**). All the variants were also examined by Sanger sequencing of PCR products amplified using genomic DNA from the five affected individuals and their parents. Nine variants were found to be false positives, 40 were inherited from either the father or mother, and 2 *de novo* heterozygous mutations of *SMARCB1* were found in 2 affected individuals (c.1130G>A (p.Arg377His) and c.1091_1093del AGA (p.Lys364del)) (**Table 1**, **Supplementary Fig. 1** and **Supplementary Methods**). Two *de novo* coding-sequence mutations occurring within a specific gene is an extremely unlikely event[8], supporting the idea that *SMARCB1* is a causative gene in CSS. Next, we screened *SMARCB1* in 23 individuals with CSS by high-resolution melting analysis[9] and identified the mutation encoding the p.Lys364del alteration in two additional individuals, including one of Arab descent (subject 22) (**Table 1** and **Supplementary Fig. 1**). As the mutation detection rate was relatively low (4 of 23, only 17.4%), we screened 15 additional genes encoding other SWI/SNF subunits (**Supplementary Table 3**). Unexpectedly, four other subunits, *SMARCA4* (also known as *BRG1*), *SMARCE1*, *ARID1A* and *ARID1B* were also found to be mutated (**Table 1** and **Supplementary Figs. 2–5**). In subject 10, a, c.2144C>T mutation in *ARID1B* (encoding p.Pro715Leu) was found in addition to the c.5632delG mutation in *ARID1B*. RT-PCR products that were amplified from total RNA from this subject's lymphoblastoid cells were cloned into the pCR4-TOPO vector. The two mutations were present on different alleles, according to sequencing of clones containing each allele (data not shown). As the c.5632delG mutation is

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Received 29 September 2011; accepted 10 February 2012; published online 18 March 2012; doi:10.1038/ng.2219

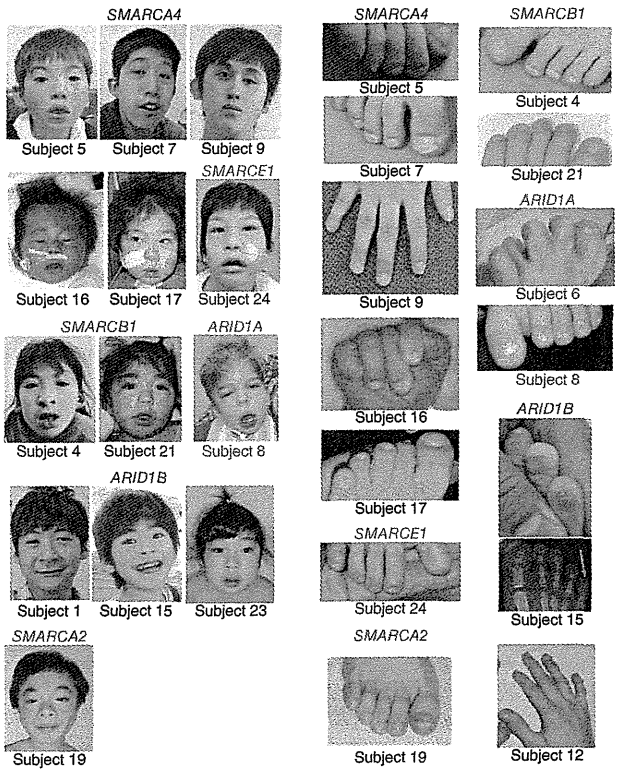

Figure 1 Photographs of individuals with Coffin-Siris syndrome. The faces (left) and hypoplastic-to-absent nail of the fifth finger or toe (right) of affected individuals are shown with the color-coded names of the corresponding mutated genes. The green arrow indicates the absence of the distal phalanx in the fifth toe. No obvious hypoplastic nails were observed in subjects 12 or 19. Consent for all the photographs was obtained from the families of the affected individuals.

very likely to be deleterious (as it results in a truncated protein), the c.2144C>T mutation is likely to be a rare polymorphism. Of note, subject 12, who presented an atypical facial appearance and indistinct hypoplastic nails, had two interstitial deletions at 6q25.3–q27 involving *ARID1B*, as detected by a SNP array (**Supplementary Fig. 6** and **Supplementary Methods**). Furthermore, subject 14 was found to have an interstitial deletion of *SMARCA2* by a SNP array (**Supplementary Fig. 7** and **Supplementary Methods**). No other copy-number changes involving genes encoding SWI/SNF complex components were found in subjects 2, 14 or 18 by array analysis. The overall mutation detection rate was 87%. In total, 20 of the 23 subjects had a mutation affecting one of the six SWI/SNF subunits.

Mutations in CSS were identified in the BAF-specific subunits *ARID1A* and *ARID1B* but not in PBAF-specific subunits (*BRD7*, *ARID2* and *PBRM1*) (**Supplementary Table 3**). In addition, mutations were identified in *SMARCA4* (*BRG1*) as well as in *SMARCA2* (*BRM*) (**Supplementary Table 3**). The BRG1 and BRM proteins are mutually exclusive catalytic ATP subunits in mammalian SWI/SNF complexes. Of note, the majority of heterozygous *Smarca4*-null mice survive with susceptibility to neoplasia, with a minority dying after birth because of exencephaly, whereas homozygous *Smarca2*-null mice are viable and fertile[4]. In *Smarca2*-null mice, Brg1 is upregulated, suggesting that Brg1 can functionally replace Brm in mice[10]. However, in humans, abnormalities in both *SMARCA4* and *SMARCA2* are found in CSS, indicating that the in-frame partial deletion of the gene encoding BRM in subject 19 has a specific mutational effect different from that of simple inactivation in mice. These data support the idea that abnormalities in the BRG1-BAF and BRM-BAF complexes can cause the abnormal neurological development in CSS.

All the mutated genes found in CSS, except for *SMARCE1*, have been reported to be associated with tumorigenesis[1,2]. Among the 23 subjects with CSS, only subject 3 with an *ARID1A* mutation presented with hepatoblastoma. To our knowledge, haploinsufficiency and/or homozygous inactivation of *ARID1A* have been found in several types of cancer but not in hepatoblastoma. Malignancies were not detected in any of the other subjects with CSS examined here. It remains to be seen whether malignancies are robustly associated with CSS.

Given the fact that all the mutations in *ARID1A* and *ARID1B* in CSS were predicted to cause protein truncation, we proposed that haploinsufficiency of these two genes must be able to cause CSS. cDNA analysis of lymphoblastoid cell lines from subjects 1, 6 and 23 indicated that the mutated transcripts were subject to nonsense-mediated mRNA decay (**Supplementary Fig. 8**). In subject 10, the *ARID1B* mutation associated with the creation of a premature stop codon in the last exon did not result in nonsense-mediated mRNA decay as expected (**Supplementary Fig. 8**).

In regard to the other mutated genes, germline heterozygous truncation mutations in *SMARCB1* and *SMARCA4* have been reported

Table 1 Mutations in individuals with Coffin-Siris syndrome

Subject ID	Gene	Mutation	Alteration	Type	Control allele frequency[a]
4	*SMARCB1*	c.1091_1093del AGA	p.Lys364del	*De novo*	0/502
11	*SMARCB1*	c.1130G>A	p.Arg377His	*De novo*	0/500
21	*SMARCB1*	c.1091_1093del AGA	p.Lys364del	NC	0/502
22	*SMARCB1*	c.1091_1093del AGA	p.Lys364del	NC	0/502
9	*SMARCA4*	c.1636_1638del AAG	p.Lys546del	*De novo*	0/350
7	*SMARCA4*	c.2576C>T	p.Thr859Met	*De novo*	0/368
5	*SMARCA4*	c.2653C>T	p.Arg885Cys	*De novo*	0/368
16	*SMARCA4*	c.2761C>T	p.Leu921Phe	*De novo*	0/368
25	*SMARCA4*	c.3032T>C	p.Met1011Thr	NC	0/372
17	*SMARCA4*	c.3469C>G	p.Arg1157Gly	*De novo*	0/368
19	*SMARCA2*	Partial deletion		*De novo*	–
24	*SMARCE1*	c.218A>G	p.Tyr73Cys	*De novo*	0/368
3	*ARID1A*	c.31_56del	p.Ser11Alafs*91	NC	0/330
6	*ARID1A*	c.2758C>T	p.Gln920*	NC	0/376
8	*ARID1A*	c.4003C>T	p.Arg1335*	*De novo*	–
1	*ARID1B*	c.1678_1688del	p.Ile560Glyfs*89	*De novo*	–
15	*ARID1B*	c.1903C>T	p.Gln635*	*De novo*	–
23	*ARID1B*	c.3304C>T	p.Arg1102*	*De novo*	–
10	*ARID1B*	c.2144C>T	p.Pro715Leu	NC	0/368
10	*ARID1B*	c.5632del G	p.Asp1878Metfs*96	NC	0/374
12	*ARID1B*	Microdeletion		NC	–

NC, not confirmed because parental samples were unavailable.
[a]The numbers indicate the observed allele frequency (alleles harboring the change/total tested alleles) in Japanese controls. None of the mutations was found in dbSNP132, the 1000 Genomes database or the National Heart, Lung, and Blood Institute (NHLBI) GO exomesequencing project database. –, not tested.

in individuals with rhabdoid tumor predisposition syndromes 1 (RTPS1; MIM 609322) and 2 (RTPS2; MIM 613325)[11,12], and various types of *SMARCB1* mutations (missense, in-frame deletion, nonsense and splice site) have been found in the germline of individuals with familial and sporadic schwannomatosis (MIM 162091)[13,14]. Furthermore, mice with heterozygous knockout of *Smarca4* or *Smarcb1* were prone to tumor development[2]. All the mutations in *SMARCA4* and *SMARCB1* in individuals with CSS were nontruncating (either missense or in-frame deletions), implying that they exert gain-of-function or dominant-negative effects (excluding haploinsufficiency as a cause). It is noteworthy that comparable germline mutations in *SMARCB1* have such different phenotypic consequences in their association with the phenotypes of CSS and schwannomatosis. The *SMARCB1* mutations in CSS and those in schwannomatosis are indeed different according to the Human Gene Mutation Database. With regard to the *SMARCA2* interstitial deletion in CSS, the change maintained the coding sequence reading frame but removed exons 20–27 that encode the HELICc domain. RT-PCR analysis confirmed the deletion of exons 20–27 at the cDNA level (**Supplementary Fig. 7**). These data suggest the importance of the HELICc domain in the SMARCA2 protein.

The various types of mutations in the genes encoding different SWI/SNF components resulted in similar CSS phenotypes. This suggests that the SWI/SNF complexes coordinately regulate chromatin structure and gene expression. This is the first report, to our knowledge, of germline mutations in SWI/SNF complex genes associated with a multiple congenital anomaly syndrome, highlighting new biological aspects of SWI/SNF complexes in humans. Similarly, genes encoding SNF2-related proteins, which are implicated as chromatin remodeling factors outside of SWI/SNF complexes, are mutated in different syndromes, including in α-thalassaemia/mental retardation syndrome X-linked (ATRX; *ATRX* mutations) and in coloboma, heart defect, atresia choanae, retarded growth and development, genital abnormality and ear abnormality (CHARGE) syndrome (*CHD7* haploinsufficiency)[3]. We expect that more mutations affecting chromatin remodeling factors will be found in different human diseases.

URLs. Human Gene Mutation Database, https://portal.biobase-international.com/cgi-bin/portal/login.cgi.

Note: Supplementary information is available on the Nature Genetics website.

ACKNOWLEDGMENTS

We thank all the family members for participating in this study. This work was supported by research grants from the Ministry of Health, Labour and Welfare (to N. Miyake, H.S. and N. Matsumoto), the Japan Science and Technology Agency (to N. Matsumoto), the Strategic Research Program for Brain Sciences (to N. Matsumoto), the Japan Epilepsy Research Foundation (to H.S.) and the Takeda Science Foundation (to N. Matsumoto and N. Miyake). This study was also funded by a Grant-in-Aid for Scientific Research on Innovative Areas (Foundation of Synapse and Neurocircuit Pathology) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (to N. Matsumoto), a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (to N. Matsumoto), a Grant-in-Aid for Young Scientists from the Japan Society for the Promotion of Science (to N. Miyake and H.S.) and a Grant for 2011 Strategic Research Promotion of Yokohama City University (to N. Matsumoto). This study was performed at the Advanced Medical Research Center at Yokohama City University. Informed consent was obtained from all the families of affected individuals. The Institutional Review Board of Yokohama City University approved this study.

AUTHOR CONTRIBUTIONS

Y.T., S. Miyatake, I.O., H.D., H.S. and N. Miyake performed exome sequencing and Sanger sequencing. Y.T., M.S., K.O., I.O., T.M., H.D., H.S. and N. Miyake performed data management and analysis. N.O., H.O., T. Kosho, Y.I., Y.H.-K., T. Kaname, K.N., H.K., K.W., Y.F., T.H., M.K., Y.H., T.Y., S.Y., S. Mizuno, S.S., T.I., T.N., T.O. and N.N. provided clinical materials after careful evaluation. Y.T., N. Miyake and N. Matsumoto wrote the manuscript. N. Matsumoto designed and oversaw all aspects of the study.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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Reply

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We thank Drs Jellinger and Attems for their interest in our study. In agreement with prior reports, we found that Parkinson disease (PD) pathology, including nigral neuronal loss and Lewy body pathology, is common in older adults without PD. Furthermore, we provide evidence that PD nigral pathology is related to parkinsonian motor signs in persons without a clinical diagnosis of PD.[1] This contrasts with prior studies of incidental Lewy body disease, which found associations with subtle electrophysiologic changes but not with overt motor signs.[2] Interestingly, in the current study, we also found that Alzheimer disease (AD) and cerebrovascular pathology showed independent associations with the severity of parkinsonian motor signs.[1] As requested, the correlations among these common brain pathologies are included in the accompanying Table. It is interesting that Dr Attems and colleagues did not find an association of nigral pathology or cerebrovascular disease with parkinsonian signs among persons with AD.[3] We and others have reported such associations.[4–6] Overall, the findings in the current study have important public health implications. They suggest that mild parkinsonian signs, reported in up to 50% of older adults by age 85 years and associated with significant morbidity and mortality, may be caused by a range of pathologies including PD pathology, AD, and cerebrovascular pathologies. These data underscore the need for more sensitive clinical measures and biomarkers that can detect and differentiate the various neuropathologies underlying the development of parkinsonian signs in old age.

Potential Conflicts of Interest

Nothing to report.

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DOI: 10.1002/ana.23639

Whole Exome Sequencing Identifies *KCNQ2* Mutations in Ohtahara Syndrome

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Recently, Weckhuysen et al revealed that *KCNQ2* mutations are involved in a substantial proportion of patients with a neonatal epileptic encephalopathy.[1] Some cases showed a suppression–burst pattern on electroencephalogram (EEG), tonic seizures, and profound intellectual disability, resembling Ohtahara syndrome (OS). By whole exome sequencing analysis of 12

TABLE: Intercorrelation of Postmortem Indices

Index	Macroinfarcts	Microinfarcts	Arteriolosclerosis	AD Pathology	Nigral Lewy Bodies
Nigral neuronal loss	0.07, 0.068	0.02, 0.628	0.13, <0.001	0.14, <0.001	0.38, <0.001
Macroinfarcts	—	0.39, 0.056	0.26, <0.001	0.09, 0.017	−0.063, 0.072
Microinfarcts		—	0.15, <0.001	0.04, 0.315	−0.10, 0.075
Arteriolosclerosis			—	0.03, 0.385	0.03, 0.491
AD pathology				—	0.07, 0.052

Based on Spearman or tetrachoric correlation and *p* value.

TABLE: Summary of the Clinical Features of Subjects with *KCNQ2* Mutations

Case #	Mutation	Sex	Age	Age at Onset, Days	Initial Symptoms	Initial Epileptic Attacks	Initial EEG	Age at Onset of Spasms, Days	Age at Onset of SB Pattern, Days	Response to Therapy	Other Drugs Used, but Ineffective	Development	Neurological Examination	Involuntary Movement
1469	c.1010C>G (p.A337G) de novo	M	7 years	7	Vomiting	7 days, tonic seizure	SB	—	22	Seizure free and SB on EEG, disappeared after high-dose PB, CPS since age 5 years	B6, ZNS	No meaningful words, able to crawl, stand with support	Severe MR, no pyramidal signs	No
1654	c.341C>T (p.T114I) de novo	F	7 years	0	Tremor of the upper extremities	2 days, generalized convulsion with cyanosis	SB	—	2	Seizure free after ZNS, CPS since age 5 years	B6, CZP, PHT	DQ 10, bed-ridden, smiling	Profound MR, spastic quadriplegia	No
1754	c.794C>T (p.A265V) de novo	M	3 months	1	Apneic spell	1 days, tonic spasms with right opsoclonuslike movement	SB	1	2	Intractable	B6, ZNS, VPA, CZP, CBZ	Delayed, no eye pursuit	Unknown	Myoclonus at the bilateral upper extremities

B6 = vitamin B6; CBZ = carbamazepine; CPS = complex partial seizures; CZP = clonazepam; DQ = developmental quotient; EEG = electroencephalogram; MR = mental retardation; PB = phenobarbital; PHT = phenytoin; SB = suppression–burst; VPA = valproic acid; ZNS = zonisamide.

patients with OS, we found 3 missense mutations in *KCNQ2* (25%): c.341C>T (p.T114I), c.1010C>G (p.A337G), and c.794C>T (p.A265V) in 3 patients. All 3 patients showed initial seizures early in the neonatal period and a characteristic suppression–burst pattern on EEG, leading to diagnosis as OS (Table). Seizures were temporarily well controlled in 2 patients. Consistent with Weckhuysen's report, in which 6 of 8 mutations arose de novo, the 3 mutations in our series are de novo changes. Thus, it is likely that de novo *KCNQ2* mutations are among the common causes of early onset epileptic encephalopathies, including OS. *KCNQ2* mutations have been shown to cause benign familial neonatal seizures, which is distinct from OS.[2,3] We unexpectedly found *KCNQ2* mutations by whole exome sequencing. Exome sequencing using familial trios (patients and their parents) can identify de novo mutations.[4] Novel associations between unexpected gene mutations and early onset epileptic encephalopathies may be validated by such new technologies.

Acknowledgment

Supported by a research grant from the Ministry of Health, Labor, and Welfare, Japan (H.S., M.K., N.Mi., N.Ma.), a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (H.S., M.K., N.Mi., N.Ma.), a research grant from the Japan Science and Technology Agency (N.Ma.), and the Strategic Research Program for Brain Sciences (a Grant-in-Aid for Scientific Research on Innovative Areas, Foundation of Synapse and Neurocircuit Pathology; N.Ma.).

Potential Conflicts of Interest

Nothing to report.

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DOI: 10.1002/ana.23620

Brain Death in Children: Why Does It Have to Be So Complicated?

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The authors appreciate the editorial comments by Wijdicks and Smith[1] and would like to address concerns about why the diagnosis of brain death in pediatric patients has to be "so complicated."

This revised clinical guideline focused specifically on determining brain death and deliberately excluded issues related to ethical concerns and organ donation. Failure to mention the Child Neurology Society (CNS) as the third sponsoring society of this guideline is a major oversight of the editorial.[1] CNS provided significant review by Practice Committee members and the society's Executive Board.[2] The quality of evidence provided in this guideline was equivalent to, if not more comprehensive than, the revised American Academy of Neurology (AAN) guideline, which reported only class III or IV evidence for 4 of 5 questions posed.[3] We used the GRADE system to develop a consensus guideline because no class I or II studies to determine pediatric brain death exist.[2] Interestingly, the AAN is currently revising guideline development for practicing neurologists to use a modification of the GRADE system.

A wide range of clinical entities can result in brain death in newborns, children, and adolescents. The guideline, the checklist, and Table 3 clearly state that all reversible conditions should be excluded prior to the first brain death examination. However, some uncertainty in the newborn period still exists leading to age-based observation periods. These consensus based recommendations reflect extensive clinical experience across several pediatric disciplines. Additionally, provisions for pediatric trauma patients and neonates were included. Virtually every committee member has cared for acutely injured children who met examination criteria for brain death within the initial 24 hours. Some recovered brain function although most did not which is why 2 examinations over defined time periods is recommended. The recommended time periods are consensus based rather than arbitrary time periods. Neurologic examination findings remaining unchanged and consistent with brain death throughout the observation period was one of the recommended criteria for determining brain death in the 1987 guidelines. The committee retained this recommendation in the current update. We agree that apparent neurologic improvements reported in anecdotal cases are due to diagnostic errors when critically examined; this is precisely the reason why a change in findings between examinations implies the neurological process is potentially reversible, precluding the diagnosis of brain death.

The revised guideline repeatedly states that brain death is a clinical diagnosis, and factors influencing the neurologic examination must be corrected before initiating brain death evaluation and apnea testing. Ancillary studies do not trump the neurological examination, and we clearly state that ancillary studies should not be viewed as a substitute for the neurologic examination. However, situations exist where ancillary studies are helpful to determine death. The revised guideline and checklist have simplified and clarified many previous sources of confusion. Additionally, the checklist will help standardize determination and documentation of brain death in children.[4]

Prolonging declaration of death does not appear to be a major concern in children—perhaps differing from the experience in adults. Families appreciate the added certainty conferred by the second examination. Patients in children's hospitals rely on assessments by pediatric specialists who understand the unique needs of children and their families. The approach to caring for children is very different and likely more family centered. These issues are further addressed in the full guideline and we encourage readers to review the entire document published in Critical Care Medicine and Pediatrics.[2,5]

Declaring brain death in children is complicated and should be undertaken by physicians who are adequately trained in the complexities involved in this important determination. We agree more research is needed to address some of the other issues raised in the editorial, and we again thank Drs Wijdicks and Smith for their opinion.

Potential Conflicts of Interest

Nothing to report.

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DOI: 10.1002/ana.23623

BRIEF REPORT

Human Mutation

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KDM6A Point Mutations Cause Kabuki Syndrome

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Communicated by Garry R. Cutting

Received 7 August 2012; accepted revised manuscript 18 September 2012.
Published online 19 October 2012 in Wiley Online Library (www.wiley.com/humanmutation). DOI: 10.1002/humu.22229

ABSTRACT: Kabuki syndrome (KS) is a rare congenital anomaly syndrome characterized by a unique facial appearance, growth retardation, skeletal abnormalities, and intellectual disability. In 2010, *MLL2* was identified as a causative gene. On the basis of published reports, 55–80% of KS cases can be explained by *MLL2* abnormalities. Recently, de novo deletion of *KDM6A* has been reported in three KS patients, but point mutations of *KDM6A* have never been found. In this study, we investigated *KDM6A* in 32 KS patients without an *MLL2* mutation. We identified two nonsense mutations and one 3-bp deletion of *KDM6A* in three KS cases. This is the first report of *KDM6A* point mutations associated with KS.
Hum Mutat 34:108–110, 2013. © 2012 Wiley Periodicals, Inc.

KEY WORDS: Kabuki syndrome; *KDM6A*; point mutations; chromosome X

Kabuki syndrome (KS; MIM# 147920), first described by Niikawa and Kuroki in 1981, is a rare congenital anomaly syndrome with the characteristic facial features of a long palpebral fissure and eversion of lateral third of the inferior eyelids [Kuroki et al., 1981; Niikawa et al., 1981]. Individuals with KS also show mild to severe intellectual disability, growth retardation, skeletal abnormalities, and a variety of visceral malformations. Although KS is thought to inherit in autosomal dominant fashion, other inheritance patterns have also been considered [Matsumoto and Niikawa, 2003]. In 2010, whole exome sequencing successfully identified loss-of-function mutations in *MLL2* in KS. *MLL2* maps to 12q13.12 and consists of at least 54 coding exons. *MLL2* encodes a histone H3 lysine 4 (H3K4)-specific methyl transferase and plays important roles in the epigenetic control of active chromatin states. On the basis of recent reports of *MLL2* mutations in KS, the mutation detection rate of *MLL2* in KS is 55–80% [Banka et al., 2012]. Among the published mutations, 73.2% (170/232) were truncation type, and pathogenic missense mutations were mainly localized in exon 48 [Banka et al., 2012].

X-linked inheritance has also been implicated in KS. Sex chromosome abnormalities in KS have been reported many times and some of the clinical manifestations are shared with Turner syndrome; patients showing overlapping features, called "Turner–Kabuki" syndrome, have been reported [Bianca et al., 2009; Dennis et al., 1993; Niikawa et al., 1988; Rodriguez et al., 2008; Stankiewicz et al., 2001; Wellesley and Slaney, 1994]. Common structural abnormalities (inversion, translocation, and ring chromosome) involving Xp11 and Yp11 in the pseudoautosomal region were observed in KS, implying the potential involvement of the regions for pathogenesis in KS [Matsumoto and Niikawa, 2003]. In addition, two unrelated KS patients with ring X (p11.2q13) have been reported [McGinniss et al., 1997; Niikawa et al., 1988]. However, an X-linked gene for KS has not been identified until recently. In 2012, complete or partial de novo deletions of *KDM6A* (MIM# 300228) were identified in three patients with KS [Lederer et al., 2012]. *KDM6A* resides at Xp11.3 and encodes the lysine demethylase 6A (KDM6A) demethylating di- and trimethyl-lysine 27 on histone H3 (H3K27) [Lee et al., 2007]. H3K4 methylation by MLL2/3 is linked to the demethylation of H3K27 by KDM6A [Lee et al., 2007]. These authors sequenced *KDM6A* in their series of 22 patients, but found no point mutations [Lederer et al., 2012]. In this study, we investigated *KDM6A* with regard to point mutations in KS after obtaining written informed consents from families of patients. The institutional review board of Yokohama City University School of Medicine approved this study.

To identify *KDM6A* mutations in KS, we examined this gene's 29 coding exons along with its exon–intron boundaries (NM_021140.2) in 32 KS individuals with no *MLL2* mutation, using high-resolution melting analysis combined with direct sequencing. We identified three mutations: c.3717G>A (p.Trp1239*) in patient 1 (male, hemizygous), c.1555C>T (p.Arg519*) in patient 2 (male, hemizygous), and c.3354_3356delTCT (p.Leu1119del) in patient 3 (female, heterozygous) (Fig. 1). Nucleotide numbering reflects cDNA numbering with +1 corresponding to the A of the ATG translation initiation codon in the reference sequence (NM_021140.2), according to journal guidelines (www.hgvs.org/mutnomen). The initiation codon is codon 1. One mutation (c.3354_3356delTCT) occurred de novo; parental samples were unavailable for the other two. Because the two nonsense mutations were outside of the last

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

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Contract grant sponsors: Ministry of Health, Labour, and Welfare (Japan) (to N.Mi., H.S., and N.Ma.); Japan Science and Technology Agency (to N.Ma.); Strategic Research Program for Brain Sciences from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (to N.Ma.); Japan Society for the Promotion of Science (to N.Mi., H.S., and N.Ma.); Strategic Research Promotion of Yokohama City University (to N.Ma.); Takeda Science Foundation (to N.Mi. and N.Ma.).

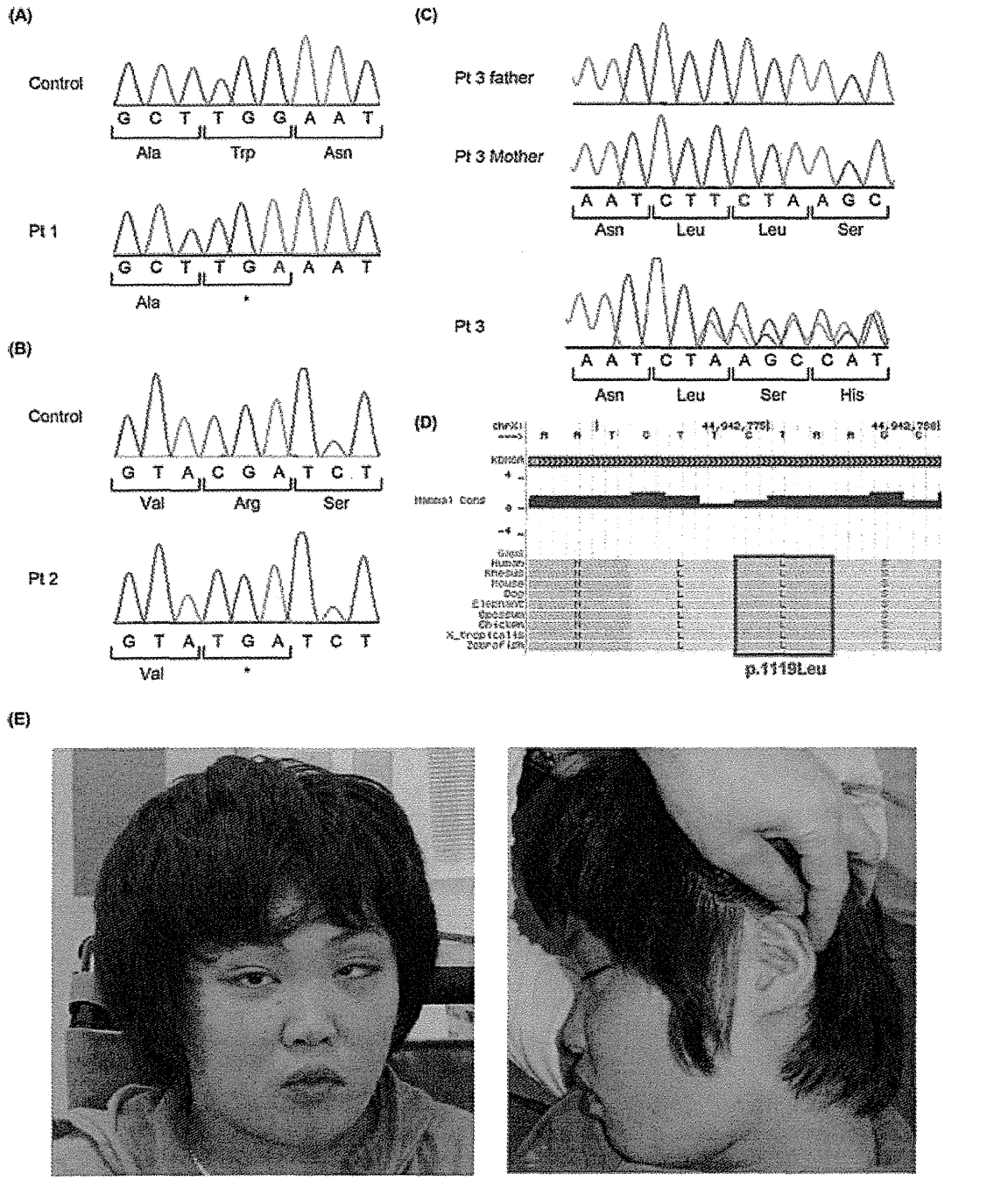

Figure 1. *KDM6A* mutations in three Kabuki syndrome patients. **A–C:** Electropherogram of patient 1: c.3717G>A (p.Trp1239*) (**A**), patient 2: c.1555C>T (p.Arg519*) (**B**), and patient 3: c.3354_3356delTCT (p.Leu1119del) (**C**). Hemizygous changes (**A** and **B**) and a heterozygous change (**C**) can be seen. The altered or deleted nucleotides are written in red. **D:** p. Leu1119 is evolutionarily conserved from zebrafish to human. The position of p.Leu1119 is boxed in red. **E:** Facial photographs of patient 3.

coding exon, and in an exon 55 bp from the 3′ most exon–exon junction, the mutant alleles could be subjected to nonsense-mediated mRNA decay (unfortunately living cells from the patients were unavailable, so we could not test this hypothesis). c.3354_3356delTCT in patient 3 would lead to deletion of one amino acid within the functionally important catalytic Jumonji C (JmjC) domain [Lee et al., 2007]. The amino residue p.Leu1119 is evolutionarily conserved from zebrafish to human (Fig. 1D) and plays an important role in hydrophobic core formation with p.Ile1126 and p.Met1129 to stabilize the JmjC domain [Sengoku and Yokoyama, 2011]. This amino acid deletion may impair helix formation around the mutated residue, resulting in domain destabilization.

Basically, *KDM6A*/*Kdm6a* escapes X-inactivation in humans and mice [Greenfield et al., 1998; Xu et al., 2008]. However, its expression from the inactive X chromosome is lower (15–35%) than that from the active X chromosome in female mice; thus, *Kdm6a* expression

Table 1. Clinical Features of Patients with a *KDM6A* Mutation

	Patient 1	Patient 2	Patient 3
Sex	Male	Male	Female
Mutation	c.3717G>A	c.1555C>T	c.3354_3356delTCT
Protein change	p.Trp1239*	p.Arg519*	p.Leu1119del
De novo status	NA	NA	De novo
Paternal age at birth	34	42	27
Maternal age at birth	33	40	26
Characteristic face	+	+	+
Microcephaly	+	+	–
Long palpebral fissures	+	+	+
Epicanthus	+	–	–
Lower palpebral eversion	+	+	+
Prominent ear	+	+	–
Auricular deformity	+	+	–
Depressed nasal tip	+	+	NA
Short nasal septum	+	+	NA
Abnormal dentition	+	+	–
Hypodontia	+	+	–
High-arched palate	+	+	–
Micrognathia	+	–	–
Short fifth finger	+	–	+
Developmental delay	+ (Severe)	+ (Severe)	+ (Severe)
Intellectual disability	+ (Severe)	+ (Severe)	+ (Severe)
Short stature	+	+	+
Prenatal growth retardation	+ (−1.96 SD)	+	–
Postnatal growth retardation	+	+	+
Cardiovascular abnormality	+	–	–
Joint laxity	+	+	–
Recurrent otitis media	+	–	–
Deafness	+[a]	–	NA
Karyotype	46,XY	46,XY	46,XX

[a]The deafness in patient 1 is conductive because of recurrent otitis media.
KDM6A gene variants were deposited in a gene-specific database (http://www.lovd.nl/KDM6A).
NA, not analyzed.

in female mice was not twice that in male mice [Xu et al., 2008]. In addition, *UTY* (Yq11.221), a paralog of *KDM6*, has been suspected to partially compensate in males while its function is not well known [Lederer et al., 2012; Xu et al., 2008]. Patient 3 in our study showed a random pattern of X-inactivation with the ratio 57:43 in genomic DNA of peripheral leukocytes. Interestingly, marked skewing of X-inactivation was observed in two female patients reported by Lederer et al. (2012). In their lymphoblast, *KDM6A* deletion was recognized at inactive X chromosome in all 70 mitoses. Here, we propose the threshold model for the pathogenicity of *KDM6A* abnormality (Supp. Fig. S1). The two female patients with a *KDM6A* deletion might not attain the appropriate level of *KDM6A* expression allowing normal development due to existence of specific cells with unfavorable inactivation, whereas male and pure Turner syndrome female with appropriate *KDM6A* expression do not show KS phenotype under assumption of unknown partial functional compensation of *KDM6A* by *UTY* in Y chromosome (only for male) (Supp. Fig. S1).

We reviewed the clinical details of the three patients (Table 1; Supp. Text). All patients were born to unrelated healthy parents. All the three showed severe developmental delay and intellectual disability. Interestingly, patient 3 (female) presented less dysmorphic features and the two male patients 1 and 2 showed a much more severe phenotype with multiple organ involvement (Table 1; Fig. 1E). Null expression of *KDM6A* in males and residual *KDM6A* expression from active X chromosome may explain sex-biased severity (Supp. Fig. S1). Alternatively, it could be explained by a lesser effect of the in-frame mutation in female patient. However, in a previous study, the severity of clinical symptoms varied also among two female patients and a male with a *KDM6A* deletion [Lederer et al., 2012]. More studies of KS patients with *KDM6A* abnormality are necessary. It is likely that the mutation type as well as the X-inactivation pattern in affected organs in females may determine the severity of KS.

In conclusion, we have described the first three point mutations of *KDM6A* in KS. Our three patients out of 32 *MLL2*-negative patients (mutation detection rate: 9.3%) are comparable to the three patients out of 22 *MLL2*-negative patients (13.6%) previously described [Lederer et al., 2012], regardless of the mutation type. The mutation detection rates for *MLL2* (55–80%) plus *KDM6A* (9–13%) in KS suggest that other gene(s) may be found. Because both MLL2 and KDM6A are histone modifiers, the other pathogenic genes might have related functions. Further research is needed to understand the pathomechanisms of KS as well as the role of histone modification in human disease.

Acknowledgments

We thank the patients and their families for participating in this work. We also thank Ms. Y. Yamashita and Ms. S. Sugimoto for technical assistance.

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