(Fig. 2F). The smaller mutant band was not detected from the mother's cDNA (Fig. 2F). Human androgen receptor assay showed that X-inactivation was random (70:30) in the mother (data not shown). However, because the percentage of mosaicism was low (20%), it remains possible that the deletion allele may undergo X-inactivation in cells possessing it, leading to diminished expression of the deletion allele in LCL.

Whole exome sequencing

To find potential pathologic mutations, whole exome sequencing of 12 patients was performed. We focused on mutations in *CASK*, and identified a hemizygous c.1A>G mutation of the first ATG codon in Patient 2 (Fig. 3A,B). This mutation is anticipated to result in alternative ATG codon usage. By using the next downstream in-frame ATG codon positioned at c.202_204 (Fig. 3C), a truncated protein without the first 67 amino acids containing calmodulin-dependent kinase domain could be produced, although this ATG codon (CATATGC) does not conform to the Kozak consensus. The parental DNA did not have the mutation, suggesting that the mutation occurred de novo (Fig. 3B). No *CASK* mutations were found in any of the other patients.

Immunoblotting

To evaluate mutational effect for CASK expression in two patients, immunoblotting was performed using total lysate of LCL. A strong signal at 104 kDa was detected in a control and the mother of Patient 1, showing strong expression of wild-type CASK protein in LCLs (Fig. 4, top). However, both Patients 1 and 2 did not show any detectable signal (Fig. 4, top), whereas the Lamin B showed comparable expression in all samples loaded (Fig. 4, bottom). Thus these data suggest that expression of CASK protein was severely decreased in two patients.

Discussion

We describe two male patients possessing an intragenic *CASK* deletion (only exon 2) or a hemizygous c.1A>G

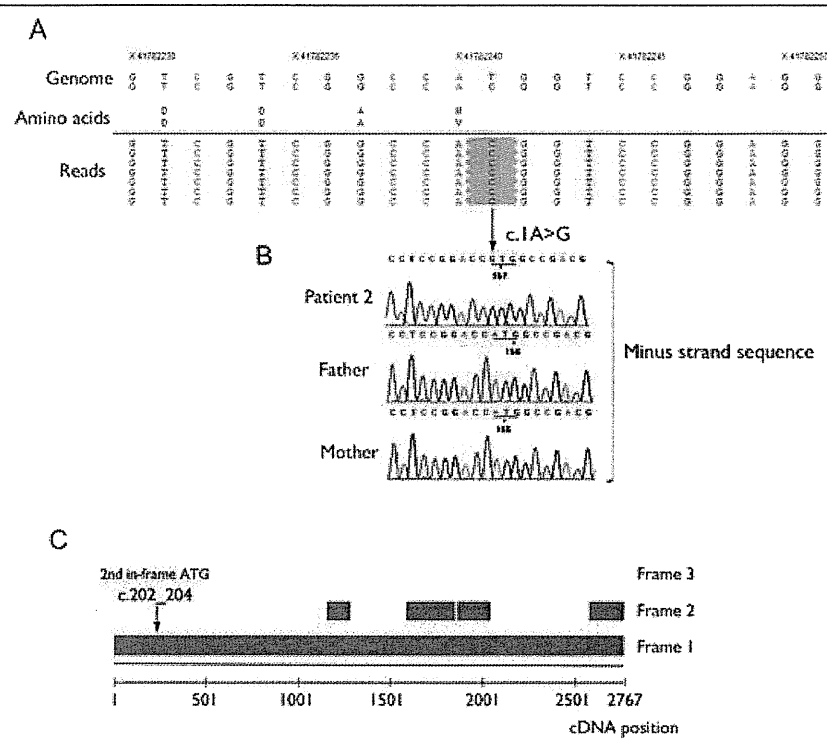

Figure 3.
c.1A>G mutation identified by exome sequencing. (**A**) From top to bottom, genomic sequence (plus strand), coding amino acids, and sequence reads covering the site of the pathogenic mutation. In genomic sequence and amino acids, upper and lower indicate reference and mutant alleles, respectively. There are six reads showing a hemizygous T>C transition at position 41,782,240 of chromosome X. (**B**) Validation of the c.1A>G mutation and inheritance analysis by Sanger sequencing. The mutation position is indicated by the arrow. (**C**) Possible open reading frames within the coding region of the *CASK* transcript (NM_003688.3). Open reading frames longer than 100 bp are shown in blue squares. The second in-frame ATG codon is positioned at c.202_204 (arrow). Any proteins longer than the protein utilizing the second in-frame ATG codon are not predicted.

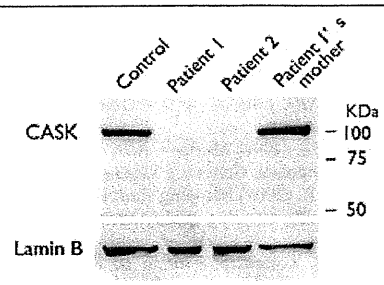

Figure 4.
Expression of CASK protein in LCL. Immunoblot analysis by using a monoclonal CASK antibody (top). Expression of CASK protein was not detected in LCL derived from two patients, whereas LCL of a control and Patient 1's mother showed strong CASK expression. The observed differences in expression were not due to difference of loading conditions, because the level of Lamin B protein was similar in all cases (bottom).

mutation. In Patient 1, the deletion is likely to be an almost null mutation as the mutant *CASK* transcript with exon 2 deletion has a frameshift with premature termination. Deletions in *CASK* have been reported in 16 female patients, and a skewed X-inactivation pattern was observed in two of them (the others had random inactivation pattern or not determined) (Froyen et al., 2007; Hayashi et al., 2008; Najm et al., 2008; Moog et al., 2011; Hayashi et al., 2012). Of interest, partial skipping of the exon 2 of *CASK* (approximately 3–6% of the unskipped transcripts) has been reported in male patients with FG syndrome showing ID, relative macrocephaly, hypotonia, severe constipation, and behavioral disturbance (Piluso et al., 2003, 2009). By contrast, our Patient 1 with complete deletion of exon 2 showed a more severe phenotype, suggesting that he showed one of the most severe phenotypes caused by *CASK* abnormalities. In Patient 2, the mutation of the first ATG codon could produce a truncated protein without the amino terminal 67 amino acids. However, this alternative in-frame ATG codon does not conform to the Kozak consensus, suggesting that its translation would be significantly reduced. In fact, CASK protein was not detected in the LCL of two patients, suggesting that expression of CASK protein should be extremely low. Because only partial skipping of exon 9 (about 20% of the mutant transcripts) (Najm et al., 2008) or of exon 2 (3–6% of the unskipped transcripts) (Piluso et al., 2009) is sufficient to cause ID and other features in male cases, it is likely that the maintenance of expression level of functional CASK protein is essential.

Two male patients with *CASK* abnormalities showed typical OS features, revealing an association between OS and *CASK* abnormalities in male patients, which has to date never been shown. Microcephaly and prominent cerebellar hypoplasia were also recognized, consistent with previous reports (Najm et al., 2008; Moog et al., 2011; Hayashi et al., 2012). Of interest, our patients also showed reduced body size and multiple congenital anomalies such as high arched palate, micrognathia, finger anomalies, and persistent hypertrophic primary vitreous. This suggests that *CASK* may be involved in overall body growth and development of these organs in humans. Supporting this idea, growth retardation and small jaw have been reported in patients with *CASK* abnormalities (Najm et al., 2008; Hackett et al., 2010; Moog et al., 2011). In addition, *CASK*-deficient mice showed micrognathia and cleft palate with male lethality (Laverty & Wilson, 1998), and hypomorphic *CASK* mutant mice are significantly smaller than littermate control mice (Atasoy et al., 2007). Therefore, it is likely that loss-of-function mutations in *CASK* cause reduced body size and multiple congenital anomalies, as well as OS and cerebellar hypoplasia.

The same deletion was found in both the mother and the affected son, indicating a germline mosaicism in the mother associated with recurrence risks. This information is useful for genetic counseling in the family. The maternal somatic mosaicism was confirmed by different methods including FISH, qPCR, and breakpoint-specific PCR analyses. We would like to emphasize the importance of breakpoint-specific PCR analysis, in which a specific band undoubtedly indicates the presence of the deletion allele. Because PCR is a powerful tool for amplifying target sequences, we could easily detect the somatic mosaic, even though it existed in approximately 20% of cells. In addition, it has been reported that PCR analyses of the deletion junction can detect extremely low-level mosaicism not detected by array comparative genomic hybridization (Zhang et al., 2009). The increasing density of available oligonucleotide arrays allows us to design long (or even regular) PCR primers for junctional cloning. Once junctional cloning is successful (though it is sometimes difficult), it is highly useful for examining parental states.

It has been determined that mutations in three genes (*STXBP1*, *ARX*, and *CASK*) cause OS. Screening for *STXBP1* mutations should be considered in OS patients with no brain anomalies in both male and female patients. Screening for *ARX* mutations would be reasonable in male patients with OS, and the presence of micropenis may encourage its screening (Kato et al., 2007). Based on this study, *CASK* mutations should be considered in patients with OS and cerebellar hypoplasia.

In conclusion, we report for the first time *CASK* abnormalities in male individuals with OS. Maternal somatic mosaicism of a *CASK* deletion is also described, suggesting that somatic and germline mosaicism of a microdeletion should be carefully considered in the examination of parental samples. Our data expand the clinical spectrum of *CASK* mutations to include OS with cerebellar hypoplasia and congenital anomalies at the most severe end of clinical presentation.

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Disclosure

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

References

Atasoy D, Schoch S, Ho A, Nadasy KA, Liu X, Zhang W, Mukherjee K, Nosyreva ED, Fernandez-Chacon R, Missler M, Kavalali ET, Sudhof TC. (2007) Deletion of CASK in mice is lethal and impairs synaptic function. *Proc Natl Acad Sci U S A* 104:2525–2530.

Djukic A, Lado FA, Shinnar S, Moshe SL. (2006) Are early myoclonic encephalopathy (EME) and the Ohtahara syndrome (EIEE) independent of each other? *Epilepsy Res* 70(Suppl. 1):S68–S76.

Froyen G, Van Esch H, Bauters M, Hollanders K, Frints SG, Vermeesch JR, Devriendt K, Fryns JP, Marynen P. (2007) Detection of genomic copy number changes in patients with idiopathic mental retardation by high-resolution X-array-CGH: important role for increased gene dosage of XLMR genes. *Hum Mutat* 28:1034–1042.

Fullston T, Brueton L, Willis T, Philip S, MacPherson L, Finnis M, Gecz J, Morton J. (2010) Ohtahara syndrome in a family with an ARX protein truncation mutation (c.81C>G/p.Y27X). *Eur J Hum Genet* 18:157–162.

Giordano L, Sartori S, Russo S, Accorsi P, Galli J, Tiberti A, Bettella E, Marchi M, Vignoli A, Darra F, Murgia A, Bernardina BD. (2010) Familial Ohtahara syndrome due to a novel *ARX* gene mutation. *Am J Med Genet A* 152A:3133–3137.

Hackett A, Tarpey PS, Licata A, Cox J, Whibley A, Boyle J, Rogers C, Grigg J, Partington M, Stevenson RE, Tolmie J, Yates JR, Turner G, Wilson M, Futreal AP, Corbett M, Shaw M, Gecz J, Raymond FL, Stratton MR, Schwartz CE, Abidi FE. (2010) *CASK* mutations are frequent in males and cause X-linked nystagmus and variable XLMR phenotypes. *Eur J Hum Genet* 18:544–552.

Hayashi S, Mizuno S, Migita O, Okuyama T, Makita Y, Hata A, Imoto I, Inazawa J. (2008) The *CASK* gene harbored in a deletion detected by array-CGH as a potential candidate for a gene causative of X-linked dominant mental retardation. *Am J Med Genet A* 146A:2145–2151.

Hayashi S, Okamoto N, Chinen Y, Takanashi JI, Makita Y, Hata A, Imoto I, Inazawa J. (2012) Novel intragenic duplications and mutations of CASK in patients with mental retardation and microcephaly with pontine and cerebellar hypoplasia (MICPCH). *Hum Genet* 131:99–110.

Hsueh YP. (2006) The role of the MAGUK protein CASK in neural development and synaptic function. *Curr Med Chem* 13:1915–1927.

Kato M, Saitoh S, Kamei A, Shiraishi H, Ueda Y, Akasaka M, Tohyama J, Akasaka N, Hayasaka K. (2007) A longer polyalanine expansion mutation in the *ARX* gene causes early infantile epileptic encephalopathy with suppression-burst pattern (Ohtahara Syndrome). *Am J Hum Genet* 81:361–366.

Kato M, Koyama N, Ohta M, Miura K, Hayasaka K. (2009) Frameshift mutations of the *ARX* gene in familial Ohtahara syndrome. *Epilepsia* 51:1679–1684.

Laverty HG, Wilson JB. (1998) Murine CASK is disrupted in a sex-linked cleft palate mouse mutant. *Genomics* 53:29–41.

Li H, Ruan J, Durbin R. (2008) Mapping short DNA sequencing reads and calling variants using mapping quality scores. *Genome Res* 18:1851–1858.

Moog U, Kutsche K, Kortum F, Chilian B, Bierhals T, Apeshiotis N, Balg S, Chassaing N, Coubes C, Das S, Engels H, Van Esch H, Grasshoff U, Heise M, Isidor B, Jarvis J, Koehler U, Martin T, Oehl-Jaschkowitz B, Ortibus E, Pilz DT, Prabhakar P, Rappold G, Rau I, Rettenberger G, Schluter G, Scott RH, Shoukier M, Wohlleber E, Zirn B, Dobyns WB, Uyanik G. (2011) Phenotypic spectrum associated with CASK loss-of-function mutations. *J Med Genet* 48:741–751.

Najm J, Horn D, Wimplinger I, Golden JA, Chizhikov VV, Sudi J, Christian SL, Ullmann R, Kuechler A, Haas CA, Flubacher A, Charnas LR, Uyanik G, Frank U, Klopocki E, Dobyns WB, Kutsche K. (2008) Mutations of CASK cause an X-linked brain malformation phenotype with microcephaly and hypoplasia of the brainstem and cerebellum. *Nat Genet* 40:1065–1067.

Nannya Y, Sanada M, Nakazaki K, Hosoya N, Wang L, Hangaishi A, Kurokawa M, Chiba S, Bailey DK, Kennedy GC, Ogawa S. (2005) A robust algorithm for copy number detection using high-density oligonucleotide single nucleotide polymorphism genotyping arrays. *Cancer Res* 65:6071–6079.

Ohtahara S, Yamatogi Y. (2006) Ohtahara syndrome: with special reference to its developmental aspects for differentiating from early myoclonic encephalopathy. *Epilepsy Res* 70(Suppl. 1):S58–S67.

Ohtahara S, Ishida T, Oka E, Yamatogi Y, Inoue H, Karita S, Ohtsuka Y. (1976) [On the specific age dependent epileptic syndrome: the early-infantile epileptic encephalopathy with suppression-burst.]. *No to Hattatsu* 8:270–279.

Piluso G, Carella M, D'Avanzo M, Santinelli R, Carrano EM, D'Avanzo A, D'Adamo AP, Gasparini P, Nigro V. (2003) Genetic heterogeneity of FG syndrome: a fourth locus (FGS4) maps to Xp11.4-p11.3 in an Italian family. *Hum Genet* 112:124–130.

Piluso G, D'Amico F, Saccone V, Bismuto E, Rotundo IL, Di Domenico M, Aurino S, Schwartz CE, Neri G, Nigro V. (2009) A missense mutation in *CASK* causes FG syndrome in an Italian family. *Am J Hum Genet* 84:162–177.

Saitsu H, Kato M, Mizuguchi T, Hamada K, Osaka H, Tohyama J, Uruno K, Kumada S, Nishiyama K, Nishimura A, Okada I, Yoshimura Y, Hirai S, Kumada T, Hayasaka K, Fukuda A, Ogata K, Matsumoto N. (2008) De novo mutations in the gene encoding STXBP1 (MUNC18-1) cause early infantile epileptic encephalopathy. *Nat Genet* 40:782–788.

Saitsu H, Kato M, Okada I, Orii KE, Higuchi T, Hoshino H, Kubota M, Arai H, Tagawa T, Kimura S, Sudo A, Miyama S, Takami Y, Watanabe T, Nishimura A, Nishiyama K, Miyake N, Wada T, Osaka H, Kondo N, Hayasaka K, Matsumoto N. (2010) *STXBP1* mutations in early infantile epileptic encephalopathy with suppression-burst pattern. *Epilepsia* 51:2397–2405.

Saitsu H, Hoshino H, Kato M, Nishiyama K, Okada I, Yoneda Y, Tsurusaki Y, Doi H, Miyake N, Kubota M, Hayasaka K, Matsumoto N. (2011) Paternal mosaicism of an *STXBP1* mutation in OS. *Clin Genet* 80:484–488.

Tarpey PS, Smith R, Pleasance E, Whibley A, Edkins S, Hardy C, O'Meara S, Latimer C, Dicks E, Menzies A, Stephens P, Blow M, Greenman C, Xue Y, Tyler-Smith C, Thompson D, Gray K, Andrews J, Barthorpe S, Buck G, Cole J, Dunmore R, Jones D, Maddison M, Mironenko T, Turner R, Turrell K, Varian J, West S, Widaa S, Wray P, Teague J, Butler A, Jenkinson A, Jia M, Richardson D, Shepherd R, Wooster R, Tejada MI, Martinez F, Carvill G, Goliath R, de Brouwer APM, van Bokhoven H, Van Esch H, Chelly J, Raynaud M, Ropers H-H, Abidi FE, Srivastava AK, Cox J, Luo Y, Mallya U, Moon J, Parnau J, Mohammed S, Tolmie JL, Shoubridge C, Corbett M, Gardner A, Haan E, Rujirabanjerd S, Shaw M, Vandeleur L, Fullston T, Easton DF, Boyle J, Partington M, Hackett A, Field M, Skinner C, Stevenson RE,

Bobrow M, Turner G, Schwartz CE, Gecz J, Raymond FL, Futreal PA, Stratton MR. (2009) A systematic, large-scale resequencing screen of X-chromosome coding exons in mental retardation. *Nat Genet* 41:535–543.

Yamatogi Y, Ohtahara S. (2002) Early-infantile epileptic encephalopathy with suppression-bursts, Ohtahara syndrome; its overview referring to our 16 cases. *Brain Dev* 24:13–23.

Zhang F, Khajavi M, Connolly AM, Towne CF, Batish SD, Lupski JR. (2009) The DNA replication FoSTeS/MMBIR mechanism can generate genomic, genic and exonic complex rearrangements in humans. *Nat Genet* 41:849–853.

Supporting Information

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

Table S1. All variants identified by exome sequencing in Patient 2.

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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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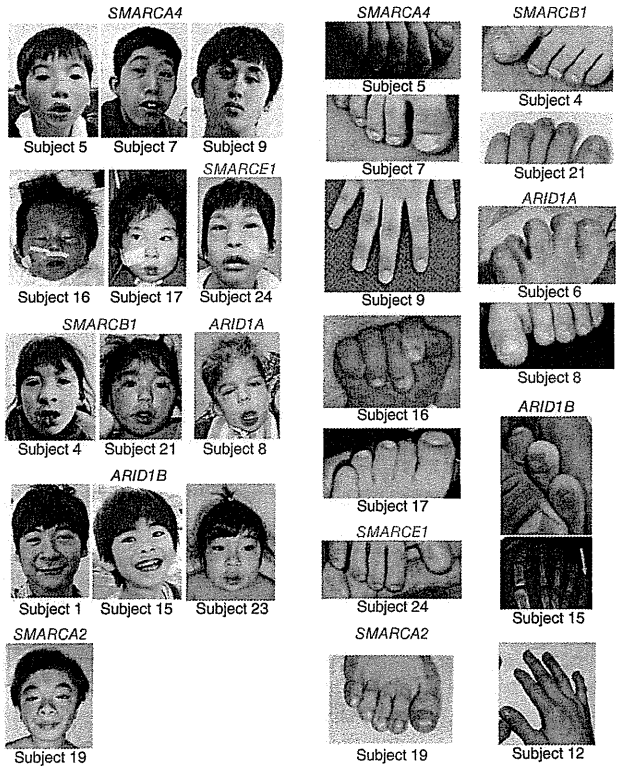

Figure 1 Photographs of individuals with Coffin-Siris syndrome. The faces (left) and hypoplastic-to-absent nail of the fifth finger or toe (right) of affected individuals are shown with the color-coded names of the corresponding mutated genes. The green arrow indicates the absence of the distal phalanx 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.

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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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1. Reisman, D., Glaros, S. & Thompson, E.A. *Oncogene* **28**, 1653–1668 (2009).
2. Wilson, B.G. & Roberts, C.W. *Nat. Rev. Cancer* **11**, 481–492 (2011).
3. Clapier, C.R. & Cairns, B.R. *Annu. Rev. Biochem.* **78**, 273–304 (2009).
4. Bultman, S. *et al. Mol. Cell* **6**, 1287–1295 (2000).
5. Hargreaves, D.C. & Crabtree, G.R. *Cell Res.* **21**, 396–420 (2011).
6. Xue, Y. *et al. Proc. Natl. Acad. Sci. USA* **97**, 13015–13020 (2000).
7. Coffin, G.S. & Siris, E. *Am. J. Dis. Child.* **119**, 433–439 (1970).
8. Bamshad, M.J. *et al. Nat. Rev. Genet.* **12**, 745–755 (2011).
9. Wittwer, C.T., Reed, G.H., Gundry, C.N., Vandersteen, J.G. & Pryor, R.J. *Clin. Chem.* **49**, 853–860 (2003).
10. Reyes, J.C. *et al. EMBO J.* **17**, 6979–6991 (1998).
11. Schneppenheim, R. *et al. Am. J. Hum. Genet.* **86**, 279–284 (2010).
12. Taylor, M.D. *et al. Am. J. Hum. Genet.* **66**, 1403–1406 (2000).
13. Boyd, C. *et al. Clin. Genet.* **74**, 358–366 (2008).
14. Hadfield, K.D. *et al. J. Med. Genet.* **45**, 332–339 (2008).