were irrelevant to the tumor ER status (the obtained ER staining results perfectly corresponded to those in patients' medical records in all cases) or 538G > A (rs178829931) polymorphism. Moreover, no correlation was found between *ABCC11* and *ESR1* expression in tumors (Pearson's correlation coefficient, $r = 0.175$) or in normal breast tissue ($r = -0.182$).

Expression of ABCC11 in BC. IHC analysis revealed an evident decrease in ABCC11 expression in tumor tissues in 8 patients as compared to the normal counterpart (Fig. 1, 1C–5C, 7C, 9C, and 10C). The quantification of green fluorescence intensity revealed 1.8- to 6.7-fold decrease of the signal (Fig. 3). ABCC11 levels in the remaining two BC samples were comparable to those in normal tissue (Fig. 1, 6C, 8C), 1.17- to 1.39-fold signal fading (see Fig. 3). Thus, none of examined tumor samples showed ABCC11 over-expression as compared to normal breast. Interestingly, in three BC samples a very low protein expression was detected despite the high mRNA levels (Fig. 1, 1C, 9C, and 10C).

DISCUSSION

In the present study we found a predominant decrease of the *ABCC11* product, ABCC11 protein, expression in BC as compared to the normal breast tissue of the same patient, and such decrease did not correlate with *ABCC11* mRNA level. Only two of ten BC specimens displayed ABCC11 expression similar to that in the normal tissue.

The function of ABCC-subfamily transporters and their role in tumor resistance are intensively investigated. *ABCC11* mRNA expression data are also available from rather numerous BC analyses. Several studies reported *ABCC11* mRNA over-expression in BC tissue and BC cell lines [8–9, 19–20]. Park *et al.* [19] observed the increased expression of *ABCC11* in BC patients with residual disease compared to those who achieved a complete response, although the authors did not include *ABCC11* in their optimal molecular prognosticator of BC response to neoadjuvant chemotherapy. Honorat *et al.* [10] pointed at the possibility of estrogen involvement in the regulation of *ABCC11* expression. On the other hand, estrogen-responsive genes have been implicated in acquired resistance to tamoxifen or aromatase inhibitors [21]. Taken together, the existing knowledge on ABCC11 expression in BC indicates that this protein may play a role in the regulation of chemotherapy response.

Most studies of tumor resistance are performed using cell cultures. However, the origin of cells used to establish cell lines does not represent all tumor types and the conditions of cell culturing appear to limit translational application of the results obtained in cell lines. Our results show that in a real tissue, *ABCC11* mRNA level poorly correlates with protein expression. This finding emphasizes the importance of parallel examination of *ABCC11* mRNA and protein product in normal and malignant breast tissue. As we demonstrated, surgical samples stored as FFPE tissues could be successfully used for such an analysis.

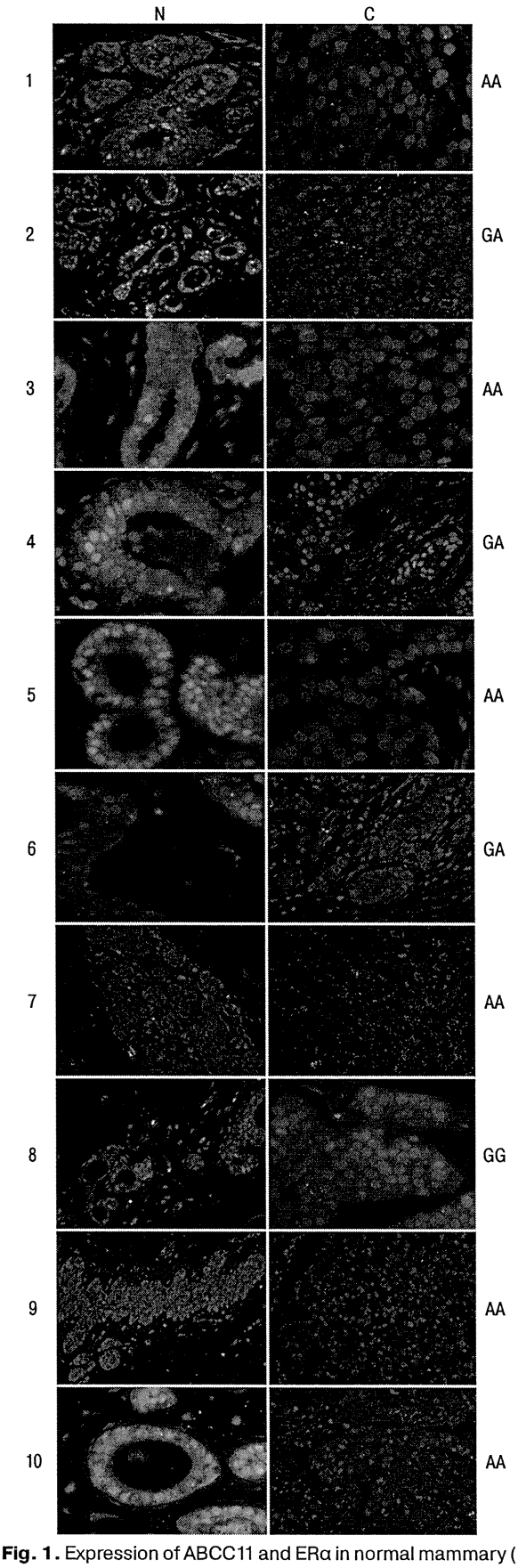

Fig. 1. Expression of ABCC11 and ERα in normal mammary (N, left column) and breast cancer (C, right column) tissues of 10 patients. The ABCC11 protein (green) and the ESR1 protein (red) were detected on 5 μm FFPE sections and merged as described in Materials and Methods. The DAPI counterstaining of the nuclei appears in blue. The genotype at rs17822931 of each patient is indicated on the right of each normal-cancer image pair

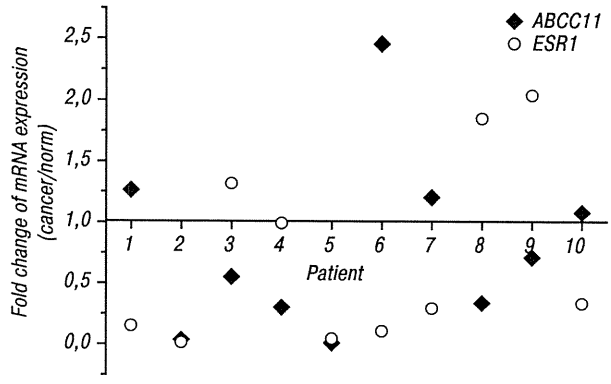

Fig. 2. Expression of *ABCC11* and *ESR1* transcripts in normal mammary tissue and breast cancer tissue of 10 patients. Expression was measured by qRT-PCR, and the geometrical mean of *ABCC11* and *ESR1* relative concentrations against three reference genes was used as an estimate of gene expression level. Black diamonds represent the ratio of *ABCC11* mRNA expression level in BC to that in normal tissue. Circles represent the changes in *ESR1* mRNA expression

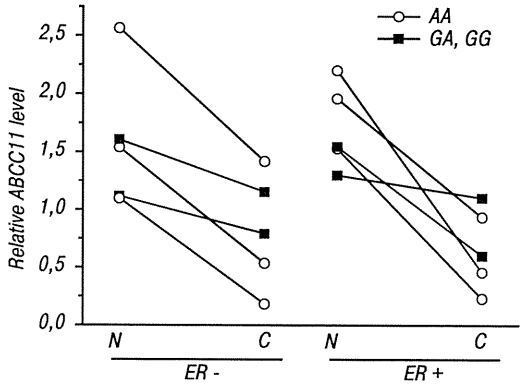

Fig. 3. Down-regulation of ABCC11 protein in BC tissues. The relative intensity of green signal was determined in normal and malignant tissue images as described in Materials and Methods. The decrease of ABCC11 fluorescence intensity from normal (N) to cancer (C) tissue was observed in each patient irrespectively of ER status or genotype

To better understand the role of ABCC11 in BC, the knowledge of protein localization in normal mammary gland is essential. Our experiments employing immunohistochemical staining demonstrated that ABCC11 is expressed in epithelial and myoepithelial cells of breast lobules and ducts. The presence of ABCC11 in epithelial cells of normal terminal duct lobular unit (TDLU), the structural and functional unit of the breast, implies the involvement of this transporter in secretion function of the mammary gland, and is consistent with the finding that the volume of colostrum secretion depends on *ABCC11* genotype at rs17822931 [12]. Of interest is the observation that ABCC11 is expressed also in myoepithelial cells which do not express ERα [22]. Our examination of ABCC11 localization may suggest that the protein participates not only in apocrine secretion, but also in metabolite transport into the stroma embedding ducts and lobules.

Transport activity of ABCC11 is strongly affected by a SNP at nucleotide 538 (538G > A, rs17822931) of *ABCC11* [14]. This SNP determines human earwax type, and associates with some functions of apocrine glands. Individuals with the AA genotype are characterized by the reduced cerumenous secretion [14] and a nearly complete loss of axillary odor [11] as compared to those homozygous or heterozygous for wild-type G allele. However, as the results reported here reveal, the *ABCC11* polymorphism does not seem to influence the localization of the ABCC11 protein in the mammary gland. The ABCC11 expression pattern was similar in the mammary glands of different *ABCC11* genotype carriers, suggesting that non-functional ABCC11 is not degraded but incorporated into the cellular membrane. Similar observations were previously made in the sweat glands [11]. Although ABCC11 expression in normal breast or BC is independent of rs17822931, functional studies of the *ABCC11* SNP are potentially useful. This could be illustrated by the study of the ABCC11 role in lung cancer cell resistance to MTA, in which ABCC11 expression level did not correlate with IC_{50} for MTA; yet *ABCC11* genotype affected chemosensitivity [6].

In conclusion, the expression of ABCC11 protein which localizes in epithelial and myoepithelial cells of normal breast lobules and ducts is likely to be decreased in the majority of BC or it may be comparable to that in normal tissue in some cases. It remains to be elucidated whether ABCC11 loss or retention in BC is functionally relevant to tumor development or may affect clinical course and prognosis. Therefore, further studies of ABCC11 expression in BC are warranted to determine its usefulness for decision making on BC therapy protocol.

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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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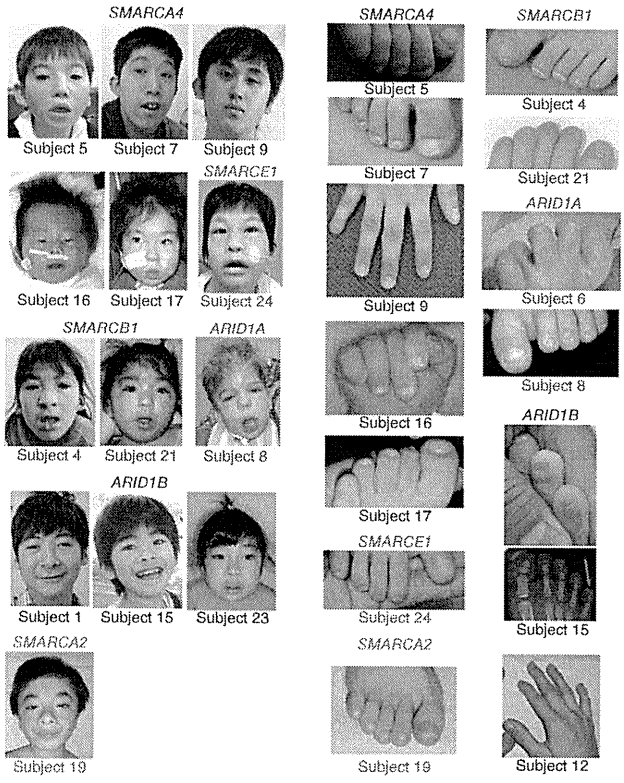

Figure 1 Photographs of individuals with Coffin-Siris syndrome. The faces (left) and hypoplastic-to-absent nail of the fifth finger or toe (right) of affected individuals are shown with the color-coded names of the corresponding mutated genes. The green arrow indicates the absence of the distal phalanx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