urologic features, but not nephrocalcinosis in our patient. Our case shared similar facial features with previous patients including a depressed nasal bridge, short anteverted nose, tented lip, and downturned corners of the mouth. Low set ears, micrognathia, malar flattening, and upslanting palpebral fissures were unique to our patient.

Hyperphosphatasia is a characteristic symptom of some GPI deficiencies, such as PIGV, PIGW, PIGO, PGAP2 and PGAP3 deficiencies [2–6]. In contrast, hypophosphatasia is a particularly distinctive feature in the loss of GPI transamidase function. Murakami at al. suggested that GPI transamidase abnormalities lead to an inability to hydrolyze the precursor protein of alkaline phosphatase, resulting in the degradation of most precursor proteins within the cell and a decrease of serum alkaline phosphatase levels (hypophosphatasia) [21]. This is supported in our case by the hypophosphatasia. The patients described by Kvarnung et al. showed hypercalcemia and hypercalciuria following tooth abnormality, craniosynostosis, a delayed bone age, and reduced mineralization, which is the common features with infantile hypophosphatasia caused by the mutations in *ALPL*, the gene encoding tissue non-specific alkaline phosphatase (TNAP) [22]. As TNAP is a GPI-AP, the PIGT deficiency causes decreased surface expression of TNAP, which would lead to bone abnormalities. Regardless of hypophosphatasia, our case showed only mild scoliosis and osteoporosis, but no tooth abnormality nor craniosynostosis. Different mutational effects on the enzyme activity may account for such different phenotypes. In this study, mutant PIGT construct harboring Arg488Trp or Glu84* in strong promoter (pME) vector restored GPI-Aps expression. In contrast, Kvarnung et al. showed that abnormal phenotype of pigt knockdown zebrafish was never restored by the homozygous mutant (Thr183Pro) PIGT cDNA. Therefore, it is possible to estimate that the Thr183Pro mutation may affect the GPI transamidase complex activity more severely than the Arg488Trp and Glu84* mutations, leading to less severe phenotypes. However, further functional analysis and cases with *PIGT* mutations are needed to elucidate the relevance of these mutations in PIGT function and full clinical spectrum of GPI deficiency syndromes.

Acknowledgments We thank the patient's family for participating in this work. We also thank Nobuko Watanabe for her technical assistance. This study was supported by the Ministry of Health, Labour and Welfare of Japan, a Grant-in-Aid for Scientific Research (A), (B), and (C) from the Japan Society for the Promotion of Science, the Takeda Science Foundation, the fund for Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program in the Project for Developing Innovation Systems, the Strategic Research Program for Brain Sciences, and a Grant-in-Aid for Scientific Research on Innovative Areas (Transcription Cycle) from the Ministry of Education, Culture, Sports, Science and Technology of Japan.

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

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American Journal of Medical Genetics Part C (Seminars in Medical Genetics) 166C:257–261 (2014)

ARTICLE

Numerous BAF Complex Genes are Mutated in Coffin–Siris Syndrome

NORIKO MIYAKE*, YOSHINORI TSURUSAKI, AND NAOMICHI MATSUMOTO

Coffin–Siris syndrome (CSS; OMIM#135900) is a rare congenital anomaly syndrome characterized by intellectual disability, coarse face, hypertrichosis, and absence/hypoplasia of the fifth digits' nails. As the majority of patients are sporadic, an autosomal dominant inheritance model has been postulated. Recently, whole exome sequencing (WES) emerged as a comprehensive analytical method for rare variants. We applied WES on five CSS patients and found two de novo mutations in *SMARCB1*. *SMARCB1* was completely sequenced in 23 CSS patients and the mutations were found in two more patients. As *SMARCB1* encodes a subunit of the BAF complex functioning as a chromatin remodeling factor, mutations in 15 other subunit genes may cause CSS and thus were analyzed in 23 CSS patients. We identified heterozygous mutations in either of six genes (*SMARCA4*, *SMARCB1*, *SMARCA2*, *SMARCE1*, *ARID1A*, and *ARID1B*) in 20 out of 23 CSS patients. The patient with a *SMARCA2* mutation was re-evaluated and identified as having Nicolaides–Baraitser syndrome (OMIM#601358), which is similar to but different from CSS. Additionally, 49 more CSS patients were analyzed as a second cohort. Together with the first cohort, 37 out of 71 (22 plus 49) patients were found to have a mutation in either one of five BAF complex genes. Furthermore, two CSS patients were reported to have a *PHF6* abnormality, which can also cause Borjeson–Forssman–Lehmann syndrome (OMIM#301900), an X-linked intellectual disability syndrome with epilepsy and endocrine abnormalities. The current list of mutated genes in CSS is far from being complete and analysis of more patients is required. © 2014 Wiley Periodicals, Inc.

KEY WORDS: Coffin–Siris syndrome; copy number analysis; target resequencing; whole exome sequencing; gene identification

How to cite this article: Miyake N, Tsurusaki Y, Matsumoto N. 2014. Numerous BAF complex genes are mutated in Coffin-Siris syndrome. Am J Med Genet Part C 166C:257–261.

INTRODUCTION

Coffin–Siris syndrome (CSS; OMIM #135900) was first reported by Dr. Coffin and Dr. Siris in 1970. The three unrelated CSS girls showed intellectual disability, postnatal growth delay, joint laxity, and short fifth digits with absent nails [Coffin and Siris, 1970]. CSS is a rare congenital disease, and approximately 100 patients were reported before gene mutations were reported [Schrier et al., 2012]. As the majority of the patients were sporadic, an autosomal dominant inheritance has been postulated. Furthermore, autosomal recessive inheritance has also been suggested, as some affected siblings have been reported [Carey and Hall, 1978; Haspeslagh et al., 1984].

In 2004, we started recruiting CSS patients for the positional cloning project. As the majority of CSS patients were sporadic, mapping of the gene was rather difficult. Disease-related chromosomal abnormalities, including submicroscopic copy number changes were the most promising clues in early 2000. Affymetrix single nucleotide polymorphism (SNP) arrays (250 K) applied on 11

Grant sponsor: Japanese Ministry of Health, Labour and Welfare; Grant sponsor: Japan Science and Technology Agency; Grant sponsor: Strategic Research Program for Brain Sciences; Grant sponsor: Grant-in-Aid for Scientific Research on Innovative Areas (Transcription cycle); Grant sponsor: Ministry of Education, Culture, Sports, Science and Technology of Japan; Grant sponsor: Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science; Grant sponsor: Grant-in-Aid for Young Scientists; Grant sponsor: Japan Society for the Promotion of Science; Grant sponsor: Grant for 2011 Strategic Research Promotion of Yokohama City University; Grant sponsor: Takeda Science Foundation.

The authors have no conflict of interest to declare.

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DOI 10.1002/ajmg.c.31406

Article first published online in Wiley Online Library (wileyonlinelibrary.com): 31 July 2014

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typical CSS patients did not reveal any abnormalities. In addition, one atypical patient showed 3.7-Mb and 5.5-Mb deletions at 6q25.3-q27, but no further investigation has been done, as their pathological significance was unknown because unavailability of parental samples interfered with de novo confirmation.

WHOLE EXOME SEQUENCING

The emerging new technology, next-generation sequencing [Shendure et al., 2004, 2008; Shendure and Ji, 2008], has become a comprehensive genetic analysis tool enabling the identification of genetic causes of unsolved Mendelian disorders [Ng et al., 2010a,b]. Especially, whole exome sequencing (WES) using genome partitioning technologies has been established and often used as a primary method for finding gene mutations causing such genetic disorders [Ng et al., 2009; Igartua et al., 2010]. As trio-based (patient, mother, and father) WES can easily identify de novo mutations, it is extremely powerful for analyzing sporadic patients with monogenic disorders showing low reproductive fitness.

We conducted WES on five sporadic CSS patients based on the hypothesis that CSS is caused by de novo mutations. For three of them, parental samples were available and trio-based analysis was done. Among 51 variants in genes commonly found in two or more patients, two de novo mutations in *SMARCB1* (SWI/SNF related, matrix associated, actin-dependent regulator of chromatin, subfamily b, member 1; isoform 1: NM_003073.3, isoform 2: NM_001007468.1) were confirmed in two patients by Sanger sequencing: one missense mutation (NM_003073.3: c.1130G > A, p.Arg377His) and one in-frame deletion (c.1091_1093delAGA, p.Lys364del) [Tsurusaki et al., 2012]. *SMARCB1* was fully screened by high resolution melting curve analysis and subsequent Sanger sequencing in 23 CSS patients including the two patients harboring the *SMARCB1* mutations. Two more patients showed an identical in-frame deletion (c.1091_1093delAGA, p.Lys364del), but their parental samples were unavailable for confirmation of the de novo occurrence. Though the detection rate of *SMARCB1* mutations was only 17.3% (4/23), two de novo mutations led us to consider that *SMARCB1* mutations indeed caused CSS. *SMARCB1* encodes SMARCB1 protein (also known as SNF5L1, BAF47, and Ini1), which is a subunit of the SWI/SNF complex that functions as a chromatin remodeling factor [Hargreaves and Crabtree, 2011; Wilson and Roberts, 2011]. As half of the subunits in the mammalian SWI/SNF complex are not present in the original yeast SWI/SNF complex, the term BAF (BRG1 and BRM-associated factor) complex is preferred in mammals [Ronan et al., 2013]. Therefore, we used BAF complex hereafter.

We conducted WES on five sporadic CSS patients based on the hypothesis that CSS is caused by de novo mutations. For three of them, parental samples were available and trio-based analysis was done. Among 51 variants in genes commonly found in two or more patients, two de novo mutations in SMARCB1 (SWI/SNF related, matrix associated, actin-dependent regulator of chromatin, subfamily b, member 1; isoform 1: NM_003073.3, isoform 2: NM_001007468.1) were confirmed in two patients by Sanger sequencing: one missense mutation (NM_003073.3: c.1130G > A, p.Arg377His) and one in-frame deletion (c.1091_1093delAGA, p.Lys364del).

CANDIDATE GENE APPROACH

Assuming that abnormalities in other BAF complex subunit genes may cause CSS, 15 other BAF complex subunit genes were screened in 23 CSS patients by HRM and Sanger sequencing: *SMARCA4* (NM_001128849.1), *SMARCA2* (NM_003070.3), *SMARCC1* (NM_003074.3), *SMARCC2* (NM_003075.3), *ARID1A* (NM_006015.4), *ARID1B* (NM_020732.3), *BRD7* (NM_001173984.2), *ARID2* (NM_152641.2), *PBRM1* (NM_018313.4), *SMARCE1* (NM_003079.4), *SMARCD1* (NM_003076.4), *SMARCD2* (NM_001098426.1), *SMARCD3* (NM_001003801.1), *ACTL6A* (NM_004301.3), and *ACTL6B* (NM_016188.4). Surprisingly, mutations in *SMARCA4*, *SMARCE1*, *ARID1A*, and *ARID1B* were found in six, one, three, and five patients, respectively [Tsurusaki et al., 2012], and the mutations were mutually exclusive. In other words, if a patient had a mutation in one of the BAF complex genes, the other genes were all normal. Retrospectively, we realized that the 3.7-Mb microdeletion previously found in the atypical patient indeed included the entire *ARID1B* gene. Furthermore, an SNP array was performed on the three patients with no apparent mutation in any BAF subunit genes and an interstitial 55-Kb deletion within *SMARCA2* was found in one patient showing atypical CSS features. We have reported on all these results as a first cohort with a mutation detection rate in the BAF complex genes of 87% (20/23) [Tsurusaki et al., 2012].

In the same issue of the journal in which we published the CSS paper [Tsurusaki et al., 2012], heterozygous *SMARCA2* mutations causing Nicolaides–Baraitser syndrome (NCBRS; MIM #601358) were reported [Van Houdt et al., 2012]. Our patient with

the interstitial deletion in *SMARCA2* was re-evaluated and diagnosed as NCBRS by professor Hennekam [Tsurusaki et al., 2013]. Therefore, excluding the NCBRS patient from our first cohort, the mutation detection rate of the BAF complex genes was 86.3% (19/22).

COMPREHENSIVE ANALYSIS

In the second cohort, targeted sequencing of 21 BAF complex genes (eight patients) or WES (44 patients) was performed on 49 new CSS patients as well as three patients without any BAF complex mutations as determined by HRM in the first cohort. The 21 genes include *ACTB* (NM_001101.3), *ACTL6A*, *ACTL6B*, *ARID1A*, *ARID1B*, *ARID2*, *BRD7*, *DPF1* (NM_001135156.2), *DPF2* (NM_006268.4), *DPF3* (NM_012074.4), *PBRM1*, *PHF10* (NM_018288.3), *SMARCA2*, *SMARCA4*, *SMARCB1*, *SMARCC1*, *SMARCC2*, *SMARCD1*, *SMARCD2*, *SMARCD3*, and *SMARCE1*. In the second cohort, we identified mutations in *SMARCA4*, *SMARCB1*, and *ARID1B* in two, three, and 15 patients, respectively [Tsurusaki et al., 2013]. Combining the two cohorts

Combining the two cohorts (excluding the NCBRS patient), we identified mutations in SMARCA4, SMARCB1, SMARCE1, ARID1A, and ARID1B in eight, seven, one, three, and 20 patients out of 71 (Fig. 1). The overall mutation detection rate was 54.9 (39/71), and 30 out of the 39 mutations were confirmed to have occurred de novo.

(excluding the NCBRS patient), we identified mutations in *SMARCA4*, *SMARCB1*, *SMARCE1*, *ARID1A*, and *ARID1B* in eight, seven, one, three, and 20 patients out of 71 (Fig. 1). The overall mutation detection rate was 54.9% (39/71), and 30 out of the 39 mutations were confirmed to have occurred de novo [Tsurusaki et al., 2013].

CSS-related mutations have been extensively analyzed by two other groups. Mutations in *ARID1A*, *ARID1B*, *SMARCA4*, *SMARCB1*, and *SMARCE1* were found by Santen et al. [2013], and mutations in *ARID1A*, *ARID1B*, *SMARCB1*, *SMARCE1*, *SMARCA2*, and *PHF6* were found by Wieczorek et al. [2013]. *SMARCA2* mutations were found in less characterized CSS/NCBRS patients; therefore, it is rather inconclusive whether *SMARCA2* mutations cause CSS [Wieczorek et al., 2013]. *PHF6* (PHD finger protein 6, NM_032458.2) mutations are rather interesting. PHF6 interacts with the nucleosome remodeling and deacetylation (NuRD) complex, which is also implicated in chromatin remodeling, but no direct interaction between PHF6 and the BAF complex has been shown [Wieczorek et al., 2013]. *PHF6* mutations are also known to cause the X-linked disorder, Borjeson–Forssman–Lehmann syndrome (OMIM *300414) [Lower et al., 2002], presenting with intellectual disability, epilepsy, and endocrinological abnormalities. In our largest cohort study, a *PHF6* mutation has never been found. It would be preferable to find more *PHF6* mutations in CSS patients for better understanding of the phenotype–genotype correlation in the *PHF6* abnormality.

The overall mutation detection rate of five BAF complex genes and *PHF6* in CSS ranged from 54.9 to 69.5% (Fig. 1) [Santen et al., 2013; Tsurusaki et al.,

The overall mutation detection rate of five BAF complex genes and PHF6 in CSS ranged from 54.9 to 69.5%. The genetic causes in the remaining patients without the BAF complex abnormality are still unknown.

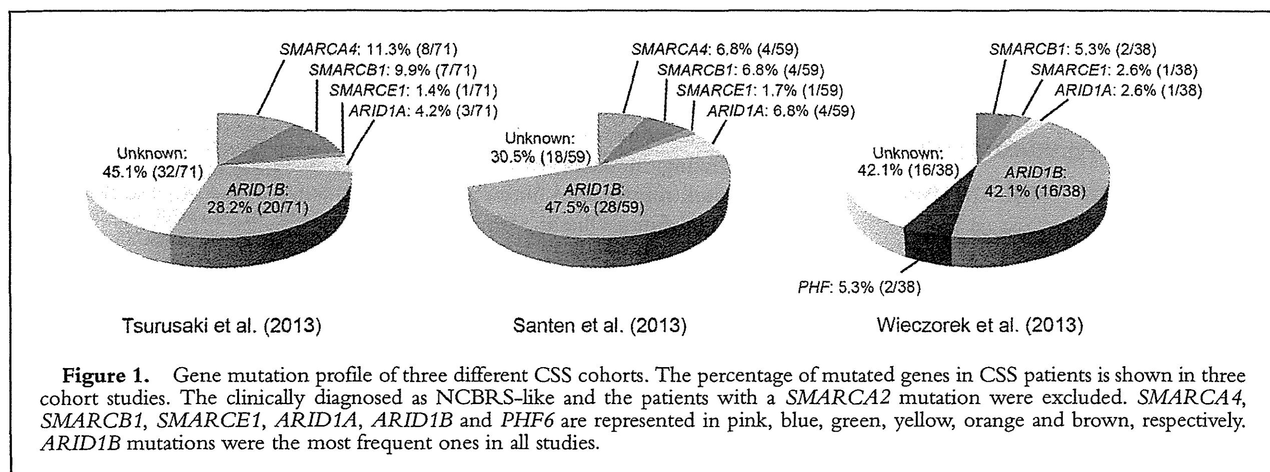

Figure 1. Gene mutation profile of three different CSS cohorts. The percentage of mutated genes in CSS patients is shown in three cohort studies. The clinically diagnosed as NCBRS-like and the patients with a *SMARCA2* mutation were excluded. *SMARCA4*, *SMARCB1*, *SMARCE1*, *ARID1A*, *ARID1B* and *PHF6* are represented in pink, blue, green, yellow, orange and brown, respectively. *ARID1B* mutations were the most frequent ones in all studies.

2013; Wieczorek et al., 2013]. The genetic causes in the remaining patients without the BAF complex abnormality are still unknown. At least three possibilities have been postulated. First, mutations in other unknown genes may contribute to CSS. Second, autosomal recessive mutations are possible. So far, six genes mutated in CSS occur mostly de novo, in an autosomal dominant fashion at the cellular level. Because some siblings affected by CSS have been reported [Coffin and Siris, 1970; Haspeslagh et al., 1984], recessive mutations may exist. Third, mutations may be missed by the current detection methods. At least, nine complete/partial gene deletions (one patient in *ARID1A* and eight patients in *ARID1B*) have been reported [Santen et al., 2012, 2013; Tsurusaki et al., 2012, 2013; Wieczorek et al., 2013], and therefore copy number analysis is recommended for complete genetic screening of CSS. In-silico copy-number variation detection programs such as exome hidden Markov model (XHMM) and copy number inference from exome reads (CoNIFER) may be recommended even in WES [Fromer et al., 2012; Krumm et al., 2012]. Eventually, whole genome sequencing together with sophisticated informatics analysis will enable us to find all the causative genetic changes in CSS.

The genotype–phenotype correlation in CSS, sparse scalp hair, abnormal/delayed dentition, ear anomalies, and absent or hypoplastic fifth phalanx of the hand are more frequently observed in mutation-positive group (mutations in *SMARCB1*, *SMARCE1*, *SMARCA4*, *ARID1A* or *ARID1B*) compared to the mutation-negative group [Tsurusaki et al., 2013]. By causative genes, *SMARCB1*, *SMARCE1*, and *ARID1A* mutations tend to cause severe intellectual disability, while *SMARCA2* and *ARID1B* mutations would cause variable severity of intellectual disability [Kosho et al., 2013]. Numbers of respective groups are still too small to make solid conclusion, further investigation would be encouraged to demarcate clinical features based on mutated genes.

The genotype–phenotype correlation in CSS, sparse scalp hair, abnormal/delayed dentition, ear anomalies, and absent or hypoplastic fifth phalanx of the hand are more frequently observed in mutation-positive group.

CONCLUSION

Mutations in BAF complex subunit genes are involved in several diseases. *ARID1B* deletion and mutations were first described in patients with intellectual disability [Hoyer et al., 2012]. Then, mutations in five BAF subunit genes were found in CSS [Tsurusaki et al., 2012] and *SMARCA2* mutations were found in NCBRS [Van Houdt et al., 2012]. Furthermore, Kleefstra syndrome and autism spectrum disorder are related to BAF complex abnormalities [Halgren et al., 2012; Santen et al., 2013; Helsmoortel et al., 2014]. Appropriate chromatin regulation by a normal BAF complex in the central nervous system is very important, especially for normal intellectual development in humans.

ACKNOWLEDGMENTS

We thank all the patients and their families for participating in this study. We are grateful to the following clinicians for their accurate clinical diagnosis, and for collecting the biological materials and clinical information: Nobuhiko Okamoto, Hirofumi Ohashi, Tomoki Kosho, Yoko Imai, Yumiko Hibi-Ko, Tadashi Kaname, Kenji Naritomi, Hiroshi Kawame, Keiko Wakui, Yoshimitsu Fukushima, Tomomi Homma, Mitsuhiro Kato, Yoko Hiraki, Takanori Yamagata, Shoji Yano, Seiji Mizuno, Satoru Sakazume, Takuma Ishii, Toshiro Nagai, Naoya Matsumoto, Yoshio Makita, Miho Adachi-Fukuda, Bertrand Isidor, Julie Perrier, Shagun Aggarwal, Ashwin B. Dalal, Adila Al-Kindy, Jan Liebelt, and David Mowat. We thank N. Watanabe, Y. Yamashita, and K. Takabe for their technical assistance. This work was supported by Research Grants from the Japanese Ministry of Health, Labour and Welfare (N. Miyake and N. Matsumoto), the Japan Science and Technology Agency (N. Matsumoto), the Strategic Research Program for Brain Sciences (N. Matsumoto), a Grant-in-Aid for Scientific Research on Innovative Areas (Transcription cycle) from the Ministry of Education, Culture, Sports, Science and Technology of Japan (N. Miyake and N. Matsumoto), a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (N. Matsumoto), a Grant-in-Aid for Young Scientists from the Japan Society for the Promotion of Science (N. Miyake), a Grant for 2011 Strategic Research Promotion of Yokohama City University (N. Matsumoto), and the Takeda Science Foundation (N. Miyake and N. Matsumoto). This study was performed at the Advanced Medical Research Center, Yokohama City University.

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