表 3　コラーゲンの遺伝子異常で発症する疾患と遺伝子異常タイプ

| コラーゲン遺伝子 | 疾患名 | 遺伝子型 | | | 計 |
| | | エクソン | | イントロン | |
		ミスセンス変異	ナンセンス変異	スプライス部位変異	
COL5A1	古典型 EDS	27.2 (6)	22.7 (5)	50 (11)	(22)
COL3A1	血管型 EDS	72.8 (107)	0.6 (1)*	26.5 (39)	(147)
COL2A1	Stickler 症候群	64.1 (82)	14.0 (18)	21.9 (28)	(128)
COL1A1	骨形成不全症	69.5 (141)	6.9 (14)	23.6 (48)	(203)
COL1A2		75.5 (105)	0.7 (1)	23.7 (33)	(139)
COL7A1	後天性表皮水疱症	56.7 (123)	19.8 (43)	24.0 (52)	(217)

表 4　EDS 各病型の診断における基準となる所見

病型	臨床症状	超微構造	コラーゲン蛋白解析	遺伝子解析	尿解析
古典型（Ⅰ/Ⅱ）	＋＋	＋＋	(＋)	＋/－	－
関節型（Ⅲ）	＋	－	－	－	－
血管型（Ⅳ）	＋＋	(＋)	＋	＋＋	－
後側彎型（ⅥA）	＋＋＋	－	＋＋	＋＋	＋＋＋
ⅦA	＋＋＋	＋＋	＋	＋＋	－
ⅦB	＋＋	＋	＋＋＋	＋＋	－
ⅦC	＋＋＋	＋＋＋	＋＋＋	＋＋	－

〔Mayer K, Kennerknecht I, Steinmann B. Clinical utility gene card for：Ehlers-Danlos syndrome types Ⅰ-Ⅶ. Eur J Hum Genet 2010（in press）より改変引用〕

質ができないため，正常の蛋白質が半分となる（haploinsuficiency）となる。従来 COL3A1 遺伝子解析は皮膚線維芽細胞の RNA 由来によるため，これらの変異を同定できなかったと考えられる。COL3A1 遺伝子解析の新たな手法が待たれるところである。

EDS の診断[8]（表 4）

　臨床症状と家族歴や類似疾患との鑑別からどの病型の Ehlers-Danlos 症候群であるかを絞り込むことが重要である。病型により，病理，蛋白解析，尿解析が確定診断に有用な場合がある。原因遺伝子が判明している場合，確定診断のために遺伝子変異解析も可能であ

る。関節可動性亢進型 EDS の診断は臨床的評価と家族歴に基づいている。常染色体優性遺伝形式を来す病型は家族歴からも遺伝形式を判定でき，家系図の作成は有用である。

おわりに

　EDS は，古典型以外は臨床医にも知られていないことが多く，時にある 1 病型や症状が代表的に印象づけられて，患者のそれぞれの多岐にわたる本来の症状・状況に合わずに対応されることが少なくない。本症候群ならびに病型の周知により，診断効率が上昇し，頻度が上がることが予測されている。近年の分子遺伝学研究の進歩で，新たな疾患関連遺伝

子が同定されている。今後，EDS は，病型内の遺伝子変異部位と臨床症状の違い，病型間での原因遺伝子ごとの症状の違いや治療効果の違いなどの知見が集積され，それぞれの病型が整理されることで，EDS の新たな分類が構築されることが望まれる。

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A novel mutation screening system for Ehlers-Danlos Syndrome, vascular type by high-resolution melting curve analysis in combination with small amplicon genotyping using genomic DNA

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ABSTRACT

Ehlers-Danlos syndrome, vascular type (vEDS) (MIM #130050) is an autosomal dominant disorder caused by type III procollagen gene (COL3A1) mutations. Most COL3A1 mutations are detected by using total RNA from patient-derived fibroblasts, which requires an invasive skin biopsy. High-resolution melting curve analysis (hrMCA) has recently been developed as a post-PCR mutation scanning method which enables simple, rapid, cost-effective, and highly sensitive mutation screening of large genes. We established a hrMCA method to screen for COL3A1 mutations using genomic DNA. PCR primers pairs for COL3A1 (52 amplicons) were designed to cover all coding regions of the 52 exons, including the splicing sites. We used 15 DNA samples (8 validation samples and 7 samples of clinically suspected vEDS patients) in this study. The eight known COL3A1 mutations in validation samples were all successfully detected by the hrMCA. In addition, we identified five novel COL3A1 mutations, including one deletion (c.2187delA) and one nonsense mutation (c.2992C>T) that could not be determined by the conventional total RNA method. Furthermore, we established a small amplicon genotyping (SAG) method for detecting three high frequency coding-region SNPs (rs1800255:G>A, rs1801184:T>C, and rs2271683:A>G) in COL3A1 to differentiate mutations before sequencing. The use of hrMCA in combination with SAG from genomic DNA enables rapid detection of COL3A1 mutations with high efficiency and specificity. A better understanding of the genotype–phenotype correlation in COL3A1 using this method will lead to improve in diagnosis and treatment.

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1. Introduction

Ehlers-Danlos syndrome, vascular type (vEDS), formerly called type IV EDS (MIM #130050) [1,2], is an autosomal dominant disorder caused by heterogeneous mutations of the gene encoding type III procollagen (COL3A1: MIM #120180) [3]. Its main clinical features are rupture of blood vessels or internal organs such as the uterus and bowel [4,5]. In the management of aneurysms, it is important to distinguish patients with vEDS due to a COL3A1 mutation from other aneurysm syndromes with FBN1 or TGFBR mutations, because tissue friability is different in these syndromes [6]. Since COL3A1 is composed of 52 exons, most COL3A1 mutations

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Abbreviations: vEDS, Ehlers-Danlos syndrome vascular type; COL3A1, type III procollagen gene; hrMCA, high-resolution melting curve analysis; SAG, small amplicon genotyping; cSNP, coding region single nucleotide polymorphism; Ct, cycle threshold; NMD, nonsense-mediated mRNA decay.
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have been detected using a reverse transcriptase PCR (RT-PCR) direct-sequencing method with total RNA extracted from patient fibroblasts, which involves an invasive skin biopsy and cell culture [5]. Therefore, we considered an alternative rapid screening method to detect COL3A1 mutations from genomic DNA.

Most mutation screening methods such as single-strand conformational polymorphism analysis [7], and denaturing high-performance liquid chromatography [8] require post-PCR manipulations, and are time consuming. A new mutation screening system termed "high-resolution melting curve analysis" (hrMCA) has recently been developed for rapid scanning of sequence variants [9–11]. The hrMCA is a closed-tube method that requires only one PCR reaction with fluorescence dye and melting analysis. Because of its ease of use, simplicity, flexibility, low cost, nondestructive nature, and high sensitivity (98%) and specificity (99.4%) [12], hrMCA is quickly becoming the tool of choice for mutation screening, especially of large genes [13].

In this study, we evaluated the hrMCA method using genomic DNA to screen the entire coding region of COL3A1. In addition, we applied detection of high frequency coding-region SNPs in

COL3A1 before sequencing, which are difficult to differentiate from mutations by hrMCA.

2. Materials and methods

2.1. Samples

We used 15 genomic DNA samples (8 validation samples with known COL3A1 mutations to validate the hrMCA, and 7 clinically suspected Japanese vEDS patients) in this study. Informed consent was obtained from each patient. Genomic DNA samples were extracted from the peripheral blood using standard procedures. Clinically suspected vEDS patients were also analyzed by using the total RNA method (RT-PCR direct-sequencing) as described previously [5].

2.2. PCR for the hrMCA method

We designed 52 PCR primer pairs covering the entire coding region of COL3A1, containing 52 exons and the adjacent exon–intron junctions, by using the GenBank genetic sequence database (GenBank ID: NM_000090.3) as the reference (Supplementary Table 1). All primers were designed with the LightScanner Primer Design software program (Idaho Technology, UT, USA). The number of domains of each amplicon was predicted by Poland's algorithm (http://www.biophys.uni-duesseldorf.de/local/POLAND/poland.html) [14]. For each amplicon, the best annealing temperature for DNA amplification was determined by using a gradient temperature of 58 °C to 72 °C in a CFX96 Real-Time PCR detection system (Bio-Rad Laboratories, CA, USA) with control genomic DNA. DNA amplification was performed for each amplicon in 96-well microtiter plates with a 10-μl final volume containing 20 ng of genomic DNA, 200 μM each of deoxynucleotide triphosphates (dNTPs), 1 × LCGreen Plus (Idaho Technology), 1 × Ex Taq buffer, 0.5 U of Ex Taq enzyme (Takara Bio, Shiga, Japan), and 0.25 μM of each primer.

The thermocycling conditions were: 2 min at 95 °C, followed by 45 cycles of amplification consisting in 30 s at 94 °C and 30 s at gradient temperature from 62 °C to 67 °C. To promote heteroduplex formation, the samples were subsequently denatured by heating to 94 °C for 30 s and cooled to 25 °C for 30 s.

2.3. hrMCA

After the PCR, the plates were imaged in a 96-well LightScanner instrument (Idaho Technology) to examine the differences in the melting curves between the patient samples and the control. The plates were heated at 0.1 °C/s, and the fluorescence was measured from 70 °C to 98 °C. The melting curves were analyzed by using the LightScanner Call IT 1.5 software program (Idaho Technology) according to the manufacturer's protocol. All melting curves deviating from the wild-type curve and appearing as a different color in different plots potentially contain a variant.

2.4. Small amplicon genotyping (SAG) for coding-region SNPs (cSNPs) in COL3A1

We used a SAG method based on hrMCA to genotype three cSNPs with high frequency in COL3A1 [15,16]. These include rs1800255:G>A, rs1801184:T>C, and rs2271683:A>G on exons 31, 33, and 49 of COL3A1, respectively. All three primer pairs were set on each exon so that the same primer pairs could be used for both genomic DNA and cDNA to detect the sequence discrepancies between genomic DNA and cDNA of the same patient (Supplementary Table 2).

DNA amplification was performed in a 96-well plate with a 10-μl final volume containing 4 μl of 2.5× high-sensitivity genotyping master mix (Idaho Technology), 1 μM of each primer, and 20 ng of genomic DNA or cDNA derived from 25 ng of total RNA. The thermocycling conditions were: 2 min at 95 °C, followed by 45 cycles of 30 s at 94 °C and 30 s at 72.6 °C (rs1801184 and rs2271683) or 74.7 °C (rs1800255). After PCR, the plate was imaged in a LightScanner and genotype identification was performed manually by using internal calibration.

2.5. Cycle sequencing

Samples from vEDS patients showing abnormal hrMCA profiles were sequenced with BigDye Terminator v3 (Applied Biosystems, CA, USA) according to the manufacturer's protocol and analyzed with ABI Prism 3130 (Applied Biosystems).

3. Results

3.1. Primer design and PCR optimization for hrMCA of COL3A1

The optimized PCR primers for the entire coding region of COL3A1 are shown in Supplementary Table 1. The amplicon length was 124–330 bp, and the best annealing temperature was between 62 °C and 67 °C. There were 45 amplicons with two domains (which are a result of a high GC region with differences within the same amplicon) and seven amplicons with one domain. The expected number of domains by Poland's algorithm and the actual hrMCA-derived number of domains were almost the same (97% correct; data not shown). To maximize the sensitivity, the fragment of exon 49 was split into two amplicons.

3.2. COL3A1 mutation screening by hrMCA

After determining the best conditions, we set up a COL3A1 mutation screening system based on hrMCA using genomic DNA. In this system, three patient samples and one control could be simultaneously analyzed in three 96-well plates (data not shown). When we started to check the differences in the melting curves between the patient samples and control by hrMCA, we obtained false positive different melting curves with the same nucleotide sequences in the same amplicon. These samples showed a large difference in Ct values, a fractional number of cycles in the PCR amplification curve of the real-time PCR, in the same amplicon detected by the real-time PCR detection instrument. The Ct value should be the same for each amplicon when the same primers are used to assay the mutant target [17]. In this study, the Ct value of each amplicon was 23–33 (mean = 25) (data not shown). Therefore, we used of all the samples with the same Ct value (Ct ± 0.7) within the same amplicon to reduce the false positives.

In eight validation samples, all mutations were correctly identified (Table 1A), including five missense (Fig. 1A–D) and three splice-site (Fig. 1H and I) mutations. Next, among clinically suspected vEDS patients, we found COL3A1 mutations in seven patients, including five novel mutations, by the hrMCA method followed by sequencing (Table 1B). The mutations included two novel missense mutations (c.539G>A; Fig. 1E, and c.2150G>A; Fig. 1F), three splice-site mutations (one novel splice-mutation at c.897+1G>C; Fig. 1G and two at c.1662+1G>A; Fig. 1I), one novel nonsense mutation (c.2992C>T; Fig. 1J) and one novel deletion mutation (1-bp) in exon 32 (c.2187delA; Fig. 1K). We also evaluated six of the seven suspected clinical samples by the total RNA method, but the nonsense mutation found by the hrMCA in genomic DNA could not be detected by the total RNA method. The deletion mutation (c.2187delA) had a frameshift, leading to a

Table 1
COL3A1 mutations detected by hrMCA of genomic DNA.

Mutation type (s)	Location	Variation	Effect	Nucleotide patterns (Wild/Mutant)		Index	Reference	Fig. 1
A. Validation samples								
Missense	Exon 18	c.1159G>T	p.G387 W	G/T		1	[5]	A
Missense	Exon 27	c.1844G>A	p.G615E	G/A		1	[5]	B
Missense	Exon 42	c.2995G>A	p.G999S	G/A		1	[4]	C
Missense	Exon 44	c.3131G>A	p.G1044D	G/A		2	[18]	D
Splice-site	Intron 20	c.1347+1 G>A		G/A		1	[5]	H
Splice-site	Intron 24	c.1662+1G>A		G/A		3[a]	[19]	I
Splice-site	Intron 24	c.1662+2 T>G		T/G		1	[20]	I

Mutation type (s)	Location	Variation	Effect	Nucleotide patterns (Wild/Mutant)	Index	Fig. 1
B. Novel mutations						
Missense	Exon 7	c.539G>A	p.G180D	G/A	1	E
Missense	Exon 32	c.2150G>A	p.G717D	G/A	1	F
Splice-site	Intron 13	c.897+1G>C		G/C	1	G
Nonsense	Exon 42	c.2992C>T	p.Q998X	C/T	1	J
Frameshift (deletion)	Exon 32	c.2187delA		A/del	1	K

Coding (c.) and protein (p.) sequences of COL3A1 are shown by the GenBank ID: NM_000090.3 with +1 corresponding to A of the ATG initiation codon.
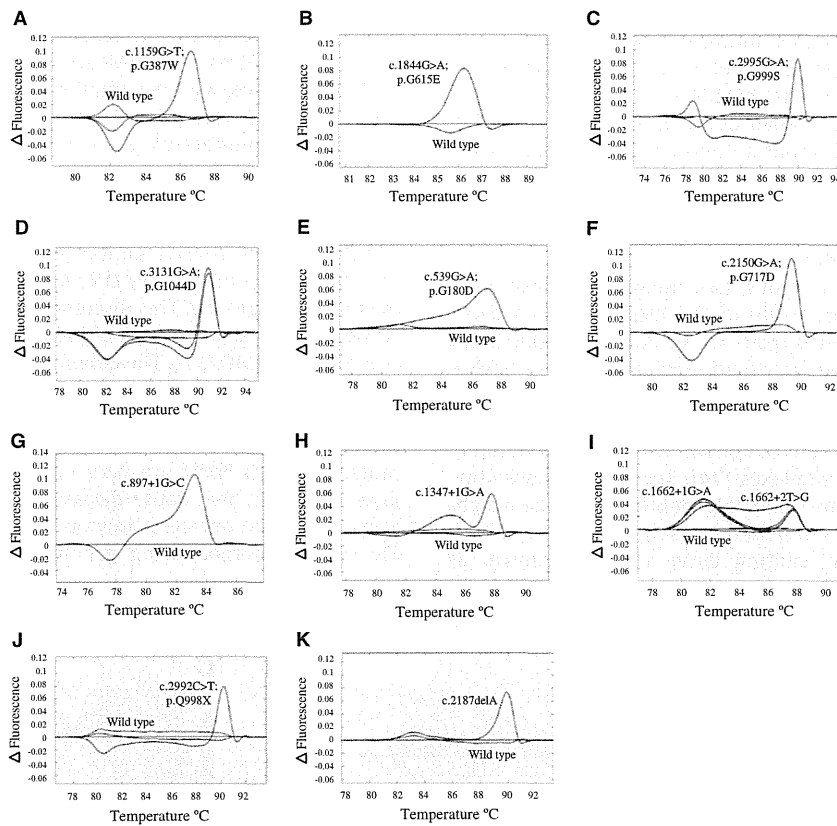[a] One sample was a validation sample and two were clinically suspected vEDS samples.

Fig. 1. Different types of COL3A1 mutations detection by the hrMCA method: missense mutations (A–F), splice-site mutations (G–I), mutations with nonsense-mediated mRNA decay; nonsense (J) and 1 bp deletion (K). Different melting curve patterns are shown between wild type and mutant COL3A1. (A) c.1159G>T in exon 18; (B) c.1844G>A in exon 27; (C) c.2995G>A in exon 42; (D) c.3131G>A in exon 44 (two different individuals); (E) c.539G>A in exon 7; (F) c.2150G>A in exon 32; (G) c.897+1G>C at intron 13; (H) c.1347+1G>A at intron 20; (I) c.1662+1G>A (three different individuals), and c.1662+2T>G (one individual) at intron 24; (J) c.2992C>T in exon 42; (K) c.2187delA in exon 32.

premature termination codon (TAG) 181 nucleotides downstream in exon 35.

Two samples from different individuals with the same mutation at c.3131G>A in exon 44 showed the same melting curve pattern (Fig. 1D). In exon 24 amplicon, four splice-site mutations (three c.1662+1G>A from different individuals and one c.1662+2T>G; Fig. 1I) were analyzed together by the hrMCA method. The three samples with the c.1662+1G>A mutation showed the same pattern, but the c.1662+2T>G sample showed a different pattern; all

melting curves were different from the control. These results showed the high sensitivity and specificity of the hrMCA method.

3.3. Heterozygous cSNPs screening by SAG

The hrMCA method could not distinguish between mutations and SNPs. Among 12 Japanese patients, we found 9 samples with one of three common cSNPs of COL3A1 (rs1800255:G>A, rs1801184:T>C, and rs2271683:A>G) by sequencing. One of these

cSNPs is present in more than half of the European or Japanese population (http://www.ncbi.nlm.nih.gov/snp/). Therefore, to predict the common heterozygous cSNPs in *COL3A1* by the SAG method is important to reduce unnecessary sequencing. By the SAG method (Supplementary Table 2), both genomic DNA and cDNA from the same patient showed the same reproducible pattern for all three cSNPs (Fig. 2A–C), and we could clearly differentiate between the wild type (homozygous) and the SNP type (heterozygous) for all three cSNPs.

When this SAG method was applied for the patient with the nonsense mutation, the results showed different melting curves between genomic DNA and cDNA at two heterozygous cSNPs (rs1801184:T>C, and rs2271683:A>G) (Fig. 2D and E), suggesting that there were nucleotide discrepancies between genomic DNA and cDNA of the same patient. In this sample, we also found sequence discrepancies between genomic DNA and cDNA at the two heterozygous cSNPs and the nonsense mutation position (c.2992C>T) by sequencing (data not shown).

4. Discussion

We successfully established a method for mutation screening of the entire coding region of *COL3A1* for diagnosis of vEDS using gradient PCR reactions and the hrMCA method for genomic DNA, followed by sequencing of abnormal hrMCA exons. By this method, *COL3A1* mutations can be screened easily and rapidly with high sensitivity and specificity without an invasive skin biopsy procedure. In addition, this system enables to detect more *COL3A1* mutations caused by nonsense-mediated mRNA decay (NMD), nonsense mutations and small deletions that could not be detected by the conventional total RNA method.

For screening *COL3A1* mutations, two important factors were noted that indicate the superiority of the hrMCA method. First, *COL3A1* mutations are heterozygous in an autosomal dominant manner, which favors higher sensitivity and specificity in hrMCA than those for homozygous mutations [21,22]. The heterozygous detection rate has nearly 100% sensitivity [23], because heterozygotes form a proportion of heteroduplexes which melt differently to perfectly matched homoduplexes [24]. Second, the nucleotide levels in *COL3A1* mutations are preferable for hrMCA because a higher difference in the melting temperature results in a greater difference between the mutant melting curve and the control as

determined by the hrMCA method [15]. The mutation patterns of G/A or T/C and G/T or A/C had a melting temperature that differed by 2 °C from the control, whereas the G/C or A/T patterns had only a 1 °C difference [12,15]. According to the reported *COL3A1* mutations, more than 85% of the missense and splice-site mutations showed G/A or G/T or T/C patterns (Human Gene Mutation Database (HGMD; http://www.hgmd.cf.ac.uk/ac/all.php)), which can be easily detected by the hrMCA method because of the greater melting temperature difference between the mutant and the wild type.

In determining the hrMCA conditions for the primer setting in each exon, the number of melting domains, which are a result of a high GC difference within the amplicon, should be considered. The number of domains for each amplicon could be predicted by Poland's algorithm before making the primers. Because hrMCA depends on the GC content of the amplicon, multiple domains can mask the presence of a mutation. Some researchers argue strongly for the use of small products, and against the use of multiple domains [25,26], but the optimal number of domains for hrMCA is still unclear [22]. In our experiment, we were able to generate the amplicon with shortest possible length in each exon containing one or two melting domains because the length of each exon of *COL3A1* is short.

It is important to reduce the false-positive results obtained by hrMCA. Samples with insufficient amplification should be reanalyzed from the PCR step in hrMCA [14]. However, it is sometimes difficult to determine whether amplification is sufficient. Therefore, we used the same Ct value (Ct ± 0.7) as a standard which is easy to evaluate, and the number of false-positive samples was reduced. For amplification of hrMCA samples, it is important to select the samples that have the same Ct value in each amplicon using a real-time PCR detection system.

In the present study, a nonsense mutation (c.2992C>T) could be detected only from genomic DNA by the hrMCA method, but not by the total RNA method. This discrepancy between genomic DNA and cDNA occurs because mRNA of the mutant allele does not mature as a result of NMD [27]. The deletion mutation (c.2187delA) in this study leads to a frameshift, causing a premature termination codon in exon 35 and is predicted not to be detected by the conventional total RNA method because of NMD [27,28]. In *COL3A1*, the reported mutations with NMD are very rare compared to other collagen gene mutations that cause diseases, because most *COL3A1* mutations have been detected previously using the total RNA method. The hrMCA method using genomic DNA in this study showed
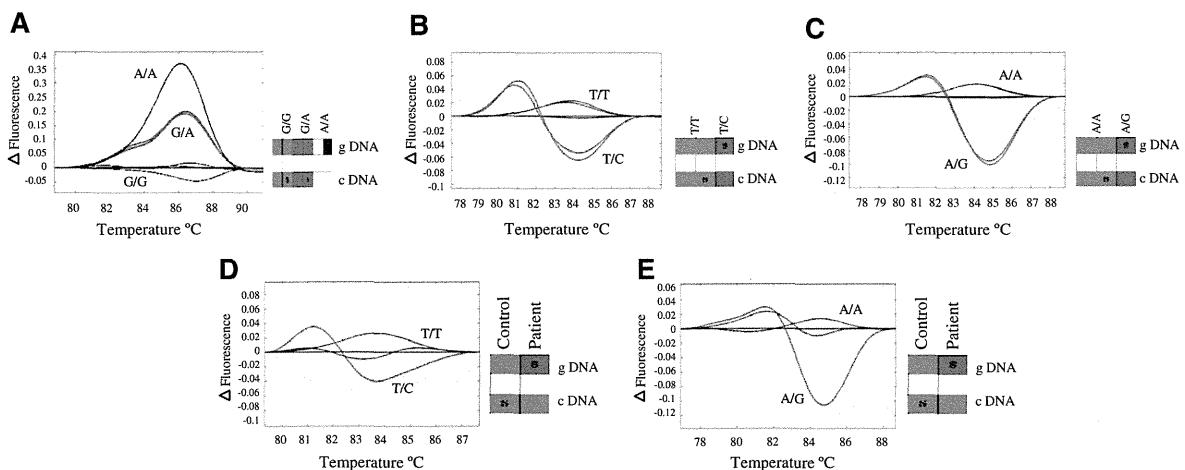

Fig. 2. *COL3A1* cSNP detection by the SAG method using genomic DNA and cDNA from the same individual: (A–C) Different melting curve patterns are shown between wild-type (homozygous) and SNP-type (heterozygous) in three common cSNPs. (A) rs1800255:G>A in exon 31 (G/G, G/A, A/A); (B) rs1801184:T>C in exon 33 (T/T, T/C); (C) rs2271683:A>G in exon 49 (A/A, A/G) genotypes. (D and E) Melting curve discrepancies between genomic DNA and cDNA are shown in *COL3A1* nonsense mutation patient compared to the control. (D) rs1801184:T>C in exon 33 and (E) rs2271683:A>G in exon 49.

higher mutation detection efficiency than the total RNA method. The number of mutations in *COL3A1*, including those caused by NMD, will likely be increased by mutation screening using genomic DNA. Screening of *COL3A1* mutations should be performed for the entire coding region of the gene by using genomic DNA for all patients showing the typical features of vEDS, regardless of whether there are negative findings based on a cDNA analysis, because nonsense mutations can occur at any position in the coding region.

Our SAG method enabled screening of the three most common cSNPs with high frequencies in *COL3A1*, and for prediction of cSNPs or mutations before sequencing. By the SAG method, nonsense mutations with any of the heterogeneous cSNPs of *COL3A1* can be predicted by checking for discrepancies between genomic DNA and cDNA. Therefore, our establishment of hrMCA for exon screening in combination with SAG of cSNPs from genomic DNA enables detection of *COL3A1* mutations with high sensitivity and specificity using a minimally-invasive procedure.

Emphasizing the importance of the new screening method, NMD has been shown to modulate the clinical outcome of genetic diseases [29]. In *COL3A1*, the phenotype severities might differ between different types of *COL3A1* mutations [28]. In the case of missense and splice-site mutations, production of the COL3A1 protein is reduced to one-eighth of the usual amount because of a dominant negative effect [2]. However, in the case of mutations with NMD, such as frame shift and nonsense mutations, the production of COL3A1 is reduced by half because of haploinsufficiency. Approaches to protect transcripts containing a premature termination codon from NMD could potentially be used as an alternative treatment [29]. A better understanding of the genotype–phenotype correlation in *COL3A1* using this method will lead to improve in the diagnosis and treatment of vEDS.

Acknowledgments

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bbrc.2011.01.011.

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Clinical Correlations of Mutations Affecting Six Components of the SWI/SNF Complex: Detailed Description of 21 Patients and a Review of the Literature

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Mutations in the components of the SWItch/sucrose nonfermentable (SWI/SNF)-like chromatin remodeling complex have recently been reported to cause Coffin–Siris syndrome (CSS), Nicolaides–Baraitser syndrome (NCBRS), and ARID1B-related intellectual disability (ID) syndrome. We detail here the genotype-phenotype correlations for 85 previously published and one additional patient with mutations in the SWI/SNF complex: four with SMARCB1 mutations, seven with SMARCA4 mutations, 37 with SMARCA2 mutations, one with an SMARCE1 mutation, three with ARID1A mutations, and 33 with ARID1B mutations. The mutations were associated with syndromic ID and speech impairment (severe/profound in SMARCB1, SMARCE1, and ARID1A mutations; variable in SMARCA4, SMARCA2, and ARID1B mutations), which was frequently accompanied by agenesis or hypoplasia of the corpus callosum. SMARCB1 mutations caused "classical" CSS with typical facial "coarseness" and significant digital/nail hypoplasia. SMARCA4 mutations caused CSS without typical facial coarseness and with significant digital/nail hypoplasia. SMARCA2 mutations caused NCBRS, typically with short stature, sparse hair, a thin vermillion of the upper lip, an everted lower lip and prominent finger joints. A SMARCE1 mutation caused CSS without typical facial coarseness and with significant digital/nail hypoplasia. ARID1A mutations caused the most severe CSS with severe physical complications. ARID1B mutations caused CSS without typical facial coarseness and with mild digital/nail hypoplasia, or caused syndromic ID. Because of the common underlying mechanism and overlapping clinical features, we propose that these conditions be referred to collectively as "SWI/SNF-related ID syndromes". © 2013 Wiley Periodicals, Inc.

Key words: Coffin–Siris syndrome; SWI/SNF complex; SMARCB1; SMARCA4; SMARCA2; SMARCE1; ARID1A; ARID1B; Nicolaides–Baraitser syndrome; intellectual disability (ID)

INTRODUCTION

Coffin–Siris syndrome (CSS; OMIM 135900) was first described by Coffin and Siris [1970]. It is a rare congenital anomaly syndrome characterized by developmental delay or intellectual disability (ID), coarse facial appearance, feeding difficulties, frequent infections, and hypoplastic-to-absent fifth fingernails and fifth distal phalanges [Devy and Baraitser, 1991; Fleck et al., 2001; Schrier et al., 2012]. We recently reported on mutations in six genes encoding components of the SWItch/sucrose nonfermentable (SWI/SNF)-like chromatin remodeling complex in 20 of 23 patients clinically diagnosed with CSS: SMARCB1 in four patients, SMARCA4 in six, SMARCA2 in one, SMARCE1 in one, ARID1A in three, and ARID1B in five [Tsurusaki et al., 2012]. In the same journal issue, truncating

mutations in ARID1B were reported in three patients with CSS and microdeletions encompassing ARID1B were reported in three patients with ID and remnants of CSS [Santen et al., 2012]. Furthermore, missense mutations of SMARCA2 were reported in 36 patients with Nicolaides–Baraitser syndrome (NCBRS; OMIM#601358) [van Houdt et al., 2012]. NCBRS was first described by Nicolaides and Baraitser [1993] and it is a recently delineated condition characterized by severe ID with absent/limited speech, seizures, short stature, sparse hair, typical facial characteristics, brachydactyly, prominent finger joints, and broad distal phalanges; the main differential diagnosis is CSS [Sousa et al., 2009]. ARID1B has also been reported to be a cause of ID. Nagamani et al. [2009] reported four patients with interstitial deletion of 6q25.2–q25.3 including ARID1B, all of whom manifested microcephaly, developmental delay, facial characteristics, and hearing impairment, and two of whom had agenesis of the corpus callosum. Halgren et al. [2012] reported eight patients with haploinsufficiency of ARID1B (de novo chromosomal translocation involving ARID1B in one, intragenic deletions in three, and microdeletion including ARID1B in four), who manifested agenesis or hypoplasia of the corpus callosum, ID with speech impairment, and autism. Hoyer et al. [2012] very recently concluded that haploinsufficiency of ARID1B is a relatively frequent cause of moderate-to-severe ID from their findings that 0.9% (8/887) of patients with unexplained ID had truncating mutations in the gene. Michelson et al. [2012] also very recently reported a patient with an interstitial 1.19 Mb deletion of 6q25.2 including ARID1B and ZDHHC14, who manifested global developmental delay, facial characteristics, dysgenesis of the corpus callosum, limb anomalies, and genital hypoplasia.

To delineate the clinical consequences of mutations affecting components of the SWI/SNF complex (CSS, NBS, and *ARID1B*-related ID syndrome), we report the individual clinical information for 20 previously reported patients [Tsurusaki et al., 2012] as well as an additional patient with an *SMARCA4* mutation. Furthermore, we create a comprehensive list of all reported patients (including our series) with mutations affecting components of the SWI/SNF complex (Tables Ia–Ic), which will be helpful when discussing similarities and differences among these conditions.

CLINICAL REPORTS
SMARCB1 Mutations

SMARCB1-1 (Subject 4 [Tsurusaki et al., 2012]; Fig. 1a–i): She was born at 42 weeks of gestation after an uncomplicated prenatal period. Her birth weight was 3,008 g (−0.5 SD). She had: cleft palate with exudative otitis media; congenital dislocation of the right hip; pectus excavatum; sucking/feeding difficulty. She underwent surgical correction of cleft palate and insertion of ventilation tubes at age 2 6/12 years. Hearing aids were required for bilateral, severe, mixed hearing impairment with a threshold of 80–90 dB. At age 4 years, she developed tonic seizures, which were treated with carbamazepine. Scoliosis, found at age 2 years, progressed with a Cobb angle of ≈150°. She showed hypotonia and motor development was severely delayed: she raised her head at age 1 8/12 years, sat alone at 2 3/12 years, and walked independently at 7 years. From ages 12 to 18 years, she vomited frequently and had recurrent infections. She had multiple dental caries, treated under general anesthesia at age 16 years. At age 21 years, she weighs 30 kg (−3.4 SD), her height is 112.5 cm (−8.4 SD), and her occipito-frontal circumference (OFC) is 51.2 cm (−2.9 SD). She understands simple commands in daily life, expresses herself with gestures, and likes to play portable personal computer games, but speaks no words. She has serious behavioral problems such as impulsiveness, hyperactivity, and self-injurious behaviors (including skin picking). She becomes exhausted easily.

SMARCB1-2 (Subject 11 [Tsurusaki et al., 2012]): Her prenatal period was complicated by intrauterine growth retardation. She was born at 38 weeks of gestation. Her birth weight was 2,088 g (−1.8 SD), length was 42 cm (−2.9 SD), and OFC was 33 cm (0 SD). She had a small ventricular septal defect (VSD). Diaphragmatic hernia was corrected surgically at age 5 months. She had sucking/feeding difficulties. She showed hypotonia and motor development was severely retarded: she rolled over at age 3 years. Generalized seizures developed and were controlled with valproic acid. Magnetic resonance imaging (MRI) of the brain showed cerebellar hypoplasia and Dandy–Walker malformation. She had visual impairment that was corrected with spectacles; hearing impairment with a threshold of 60 dB in the right ear and 50 dB in the left ear was noted. At age 7 years, her weight is 12 kg (−3.1 SD) and height is 105 cm (−2.7 SD). She sits for several seconds with her hands, distinguishes her family members from others, and smiles when called by her name. She has visual and hearing impairment.

SMARCB1-3 (Subject 21 [Tsurusaki et al., 2012]; Fig. 1j–p): Her prenatal period was complicated by intrauterine growth retardation and oligohydramnios. She was born at 38 weeks of gestation, followed by resuscitation through endotracheal intubation. Her birth weight was 1,746 g (−2.6 SD). Surfactant treatment was undertaken for pulmonary hemorrhage. She had micrognathia, exotropia, and a dark complexion. She suffered from complex partial seizures. She sucked poorly and then had feeding difficulty associated with gastroesophageal reflux (GER), which required gastrostomy. She showed hypotonia with severe delay in motor development. A hypoplastic corpus callosum was observed. At age 7 years, she weighs 12 kg (−3.0 SD), has a height of 97 cm (−4.5 SD), and an OFC of 44 cm (−5.1 SD). She is unable to sit alone, and moves by rolling over. She cannot communicate with others or speak any words, but smiles when she appears to be happy.

SMARCB1-4 (Subject 22 [Tsurusaki et al., 2012]): He was born at 37 weeks of gestation. His birth weight was 2,784 g (+0.2 SD). He was admitted to hospital as a newborn for treatment of transient tachypnea. He had pyloric stenosis that was corrected surgically. He sucked poorly and then had feeding difficulties associated with GER, requiring gavage-feeding and resulting in failure to thrive. He showed hypotonia and motor development was severely delayed. MRI of the brain showed hypoplasia of the corpus callosum. He suffered from recurrent respiratory tract infections. At age 2 years, he weighed 9.7 kg (−2.3 SD), his height was 83.4 cm (−2.2 SD), and had an OFC of 43 cm (−3.3 SD). At age 3 years, he rolls over, but cannot sit alone or speak words.

SMARCA4 Mutations

SMARCA4-1 (Subject 9 [Tsurusaki et al., 2012]; Fig. 2a–c): He was born at 39 weeks of gestation. His birth weight was 2,880 g. Sucking or feeding difficulties were not reported, but abdominal distension occurred in infancy and constipation was frequent in childhood. Possible seizures developed once at age 1 2/12 years with unconsciousness but without electrocardiographic abnormalities. The submucosal cleft palate was corrected surgically at age 1 6/12 years. Congenital torticollis with vestigial (right) and shortened (left) cleidomastoid muscles was corrected surgically. His right chest was funnel-shaped with a hypoplastic right pectoral major muscle. Exudative otitis media in the left ear was recurrent. He showed hypotonia in his infancy and motor development was mildly delayed: he raised his head at age 4 months, sat alone at 10 months, crawled at 11 months, and stood alone at 1 3/12 years. His hair has been bristly and gray-streaked since childhood. His upper teeth were misaligned. At age 18 years, his height is 159 cm (−1.8 SD) and OFC is 53 cm (−2.3 SD). He can walk independently, talk (albeit with a stutter), and understand almost everything necessary for daily life. He has myopia and mild astigmatism which are corrected with spectacles. He has nocturnal enuresis which he finds hard to control.

SMARCA4-2 (Subject 7 [Tsurusaki et al., 2012]; Fig. 2d–g): His prenatal period was complicated by intrauterine growth retardation. He was born at 40 weeks of gestation. His birth weight was 2,250 g (−2.2 SD). He showed respiratory insufficiency and had sucking/feeding difficulties associated with laryngomalacia. He had bilateral ptosis, myopia, lacrimal duct stenosis, bilateral sensorineural hearing loss, and ankyloglossia. He showed hypotonia and motor development was severely delayed: he raised his head in late infancy, sat alone at age 2 years, and walked independently at 6 years. At age 20 years, he weighs 60 kg (−0.2 SD), has a height of

TABLE IA. Clinical Features of Patients With Mutations in the Components of SWI/SNF Complex

Gene	SMARCB1				SMARCA4						
Patient (subject no. in the previous report§)	1 [4]	2 [11]	3 [21]	4 [22]	1 [9]	2 [?]	3 [5]	4 [16]	5 [25]	6 [17]	7
Age at publication (years of age)	21	?	?	2	18	20	9	11	16	4	8
Sex	F	F	F	M	M	M	M	M	F	M	F
Mutation	p.Lys364del	p.Arg377His	p.Lys364del	p.Lys364del	p.Lys546del	p.Thr859Met	p.Arg885Cys	p.Leu921Phe	p.Met1011Thr	p.Arg1157Gly	p.Arg885His
Growth											
Prenatal growth (birth weight/length)#	−0.5/?	−1.8/−2.9	−2.6/?	+0.2/?	—	−2.2/?	−1.2/−0.9	−1.7/−1.6	−1.0/−1.9	−1.1/−2.3	−2.6/−2.7
Postnatal growth (weight/height)†	−3.4/−8.4	−3.1/−2.7	−3.0/−4.5	−2.3/−2.2	?/−1.8	−0.2/−2.6	−1.5/−3.2	−1.8/−3.1	−1.9/−1.9	−3.0/−3.4	−1.9/−1.8
Psychomotor											
Developmental delay/intellectual disability‡	Severe	Severe	Severe	Severe	Mild	Severe	Severe	Severe	Severe	Severe	Moderate
Speech delay	NW	NW	NW	NW	Mild	SW	NW	NW	SC	NW	Mild
Seizures	+	+	—	—	+	—	—	+	—	—	—
Hypotonia	+	+	+	+	+	—	+	—	—	—	+
Autistic features/behavioral abnormalities	HA,Im,SH		—			HA, Im	ASD (HA, HS, Ob, SH)	—	RB		HA
Microcephaly (≤2 SD) (SD score)	+(−2.9)	—	+(−5.1)	+(−3.3)	+(−2.3)	+(−3.8)	+(−3.6)	+(−2.9)	+(−2.3)	+(−2.7)	+(−3.0)
Brain anomaly		CH,DW	HCC	HCC			HCC,HCV			HCC	—
Craniofacial											
Sparse hair	+	+	+	+	—	+	—	+	—	+	—
Thick eyebrows	+	+	+	+	+	+	+	+	—	+	+
Thick eyelashes	+	+	+	+	+	+	+	+	+	+	+
Ptosis	—	+	+	+	+	+	+	+	—	+	+
Abnormal ears	+	+	+	+	—	+	+	+	+	+	—
Nasal bridge	Broad	Broad	Broad	Broad	Narrow	Narrow	Normal	Flat	Flat	Flat	Flat
Thick, anteverted alae nasi	—	—	—	+	—	—	—	—	—		
Wide mouth	+	+	+	+	—	—	+	—	+	—	+
Philtrum	Broad		Long	Long	Short	Short	Short		Short		Short
Upper lip vermilion feature	Thin		Thin	Thick	Everted	Everted	Everted	Thin	Everted	—	
Thick lower lip vermilion	+	+	+	+	+	+	+	+	+	+	+
Palatal abnormality	C	C	H	H	SMCP	H	C	H	H	C	H
Skeletal–limb											
Hypoplastic/absent fifth finger/toe	Fi/T	Fi/T	Fi/T	Fi/T	Fi	Fi/T	T	Fi/T	Fi/T	Fi/T	T
Hypoplastic/absent nail (fifth finger/toe)	Fi/T	+	Fi/T	Fi/T	Fi	Fi/T	T	Fi/T	Fi/T	Fi/T	T
Hypoplastic/absent nail (other fingers/toes)	Fi/T		Fi/T	Fi/T		Fi/T	T	Fi/T	Fi/T	Fi/T	—
Prominent interphalangeal joints	+		—	—	—	+	—	—	+	—	
Prominent distal phalanges	+	+	—	—	+	+	+	+	+	—	
Scoliosis/spinal abnormalities	+		+	+	—	+	—	—	—	—	+
Joint laxity		—	+	+	—	+	+	—	+	—	+
Others											
Hirsutism	+		+	+	+	+	+	+	+	+	+
Congenital heart defects	—	VSD	—	+	—	—	—	VSD, PDA	—	MA, PA, SRV, AtSD, PDA	+
Genitourinary defects	—			+			Cr	—	—	Cr	—
Gastrointestinal abnormalities	—	—	GER	PS		GOO	Co	DU	Co	GER	GER
Inguinal (I)/umbilical (U) hernia	—	—	I	I	—	I	—	I	—	Om	I/U
Sucking difficulty	+	+	+	+		+	+	+	+	+	+
Feeding difficulty	+	+	+	+	—	+	+	+	+	+	+
Frequent vomiting	+										+
Hearing impairment	+	+	+		—	+	+		+	—	—
Visual impairment		+	+		+	+	+	+	+	—	—
Recurrent infections	+	—	+	+		+	—		+	+	+

+, present; —, absent; blank, data not available; §, Tsurusaki et al. [2013]; #, SD score; †, SD score; ‡, at latest assessment; ASD, autism spectrum disorder; AtSD, atrial septal defect; C, cleft palate; CH, cerebellar hypoplasia; Co, constipation; Cr, cryptorchidism; DU, duodenal ulcer; DW, Dandy-Walker malformation; F, female; Fi, finger; GER, gastroesophageal reflux; GOO, gastric outlet obstruction; H, high palate; HA, hyperactivity; HCC, hypoplastic corpus callosum; HCV, hypoplastic cerebellar vermis; HS, hypersensitivity; I, inguinal; Im, impulsiveness; M, male; MA, mitral atresia; NW, no words; Ob, obsession; Om, omphalocele; PA, pulmonary atresia; PDA, patent ductus arteriosus; PS, pyloric stenosis; RB, repetitive behavior; SC, simple conversation; SH, self-harming behavior; SMCP, submucosal cleft palate; SRV, single right ventricle; SW, several words; T, toe; VSD, ventricular septal defect

TABLE IB. Clinical Features of Patients With Mutations in the Components of SWI/SNF Complex

Gene	SMARCA2		SMARCE1	ARID1A			ARID1B			
Patient [subject no. in the previous report§]	1 [19]	NCBRS[n = 36]	1 [24]	1 [3]	2 [6]	3 [8]	1 [1]	2 [15]	3 [23]	4 [10]
Age at publication (years of age)	8	2 3/12–32	14	2	1	10	13	7	10	11
Sex	M	21M,15F	F	M	M	M	M	M	F	M
Mutation	Partial deletion [van Houdt et al., 2012]	Missense	p.Tyr73Cys	p.Ser11Alafs*91	p.Gln920*	p.Arg1335*	p.Ile560Glyfs*89	p.Gln635*	p.Arg1102*	p.Asp1878Metfs*96
Growth										
Prenatal growth (birth weight/length)#	−2.6/−3.1	10/34	−2.3/?	−1.0/−1.4	−0.6/−1.1	—	−0.9/+0.4	−1.2/−1.2	+0.5/−1.7	−1.0/−1.1
Postnatal growth (weight/height)†	−2.1/−3.8	19/36	−4.4/−6.8	−6.2/−8.9	−5.5/−8.2	50th/3rd	−0.5/−1.4	−1.1/−1.1	+1.5/−1.3	−0.5/−2.4
Psychomotor										
Developmental delay/intellectual disability‡	Moderate	3Mild,9Moderate,24Severe	Severe	Severe	Severe	Severe	Moderate	Severe	Mild	Mild
Speech delay	NW	13/33NW	NW	NW	NW	NW	NW	NW	Mild	Mild
Seizures	+	22/35	+	−	−	−	−	+	+	−
Hypotonia	+		+	+	+	−	−	+	+	+
Autistic features/behavioral abnormalities	−					HA	ASD [HA, Im, O]	ASD [Im]	−	
Microcephaly (≦2 SD) (SD score)	+(−3.3)	19/34	+(−6.7)	+(−3.7)	+(−3.1)	−(50th)	−(+0.7)	−(−1.4)	−(+1.8)	−(−1.1)
Brain anomaly				ACC, CH,DW	ACC	HCC			ACC, Colp	
Craniofacial										
Sparse hair	−	35/36	+	+	+	+	+	−	−	+
Thick eyebrows	+		+	+	+	+	+	+	+	+
Thick eyelashes	+	23/35	+	+	+	+	+	+	+	+
Ptosis	+		+	−	−	−	−	−	−	−
Abnormal ears	+		+	+	+	+	+	+	−	+
Nasal bridge	Broad	22/36Narrow	Narrow	Broad	Broad		Broad	Broad	Broad	Broad
Thick, anteverted alae nasi	+	32/36	−		−	−	−	+		
Wide mouth	+	34/36	+	+	+	+	+	+	−	+
Philtrum	Broad	31/36Broad, 29/36Long	Long	Short	Broad	Long	Broad	Broad	Long	Long
Upper lip vermilion feature		27/36Thin			Everted		Thin	Thick	Thin	Thin
Thick lower lip vermilion	+	32/36	+	+	+	+	−	+	+	+
Palatal abnormality	−		C	C	C	H	H	H	H	H
Skeletal—limb										
Hypoplastic/absent fifth finger/toe	−		Fi/T		Fi/T	Fi/T	Fi/T	Fi/T	Fi	Fi/T
Hypoplastic/absent nail (fifth finger/toe)	−		Fi/T	+	Fi/T	Fi/T	Fi/T	T	Fi	Fi/T
Hypoplastic/absent nail (other fingers/toes)	−		Fi/T	Fi/T		Fi/T	Fi/T	T		Fi/T
Prominent interphalangeal joints	+	28/35	−		−	−	−	−	−	+
Prominent distal phalanges	+	21/35	−		−	+	+	+	−	+
Scoliosis/spinal abnormalities	+	10/34		+	+	−	+	−	−	+
Joint laxity	−			+	+	−	+	+		+
Others										
Hirsutism	+		+	+	+	+	+	+	+	+
Congenital heart defects	−	6/34	TR,MR,AS	CoA, AtSD, VSD	CoA, AS, VSD	AtSD	−	−	−	+
Genitourinary defects	Cr, HS	13/20Cr,2/32VUR	−		HS, Cr	−	−	−	−	Cr
Gastrointestinal abnormalities					AA, RUF	IO, GER				
Inguinal (I)/umbilical (U) hernia	I/U	14/35	−	−	−	I	−	−	−	−
Sucking difficulty	+		+	+	+	+	+	+	+	+
Feeding difficulty	+		+	+	+	+	+	+	+	+
Frequent vomiting	+									
Hearing impairment	+			+	−	+		+	−	−
Visual impairment	+		−	−			+	−	+	−
Recurrent infections	−	+	+	+	+	+	+	+	+	+

+, present; −, absent; blank, data not available; §, Tsurusaki et al. [2013]; #, SD score;†, SD acore; ‡, at latest assessment; AA, anal atresia; ACC, agenesis of corpus callosum; AS, aortic stenosis; ASD, autism spectrum disorder; AtSD, atrial septal defect; C, cleft palate; CH, cerebellar hypoplasia; CoA, coarctation of aorta; Colp, colpocephaly; Cr, cryptorchidism; DW, Dandy-Walker malformation; F, female; Fi, finger; GER, gastroesophageal reflux; H, high palate; HA, hyperactivity; HCC, hypoplastic corpus callosum; HS, hypospadias; Im, impulsiveness; IO, intestinal obstruction; M, male; MR, mitral regurgitation; NBS, Nicolaides-Baraitser syndrome; NW, no words; O, obsession; RUF, retrourethral fistula; T, toe; TR, tricuspid regurgitation; VSD, ventricular septal defect; VUR, vesicoureteral reflux; fractions in the previous publications show patient numbers "feature-positive/data available"

TABLE IC. Clinical Features of Patients With Mutations in the Components of SWI/SNF Complex

Gene				ARID1B		
Patient (subject no. in the previous report§)	5 (12)	n = 4	n = 8	n = 9	n = 6	n = 1
Age at publication (years of age)	19	11/12–4	3–46	3 3/12–20	2–40	2
Sex	M	2M,2F	2M,6F	4M,5F	1M,5F	M
Mutation	Del (9.2Mb)	Del (3.77–13.81Mb) [Nagamani et al., 2009]	7Del (0.2–14.5Mb),1Tra [Halgren et al., 2012]	1Del(2.5Mb),1Dup(exon 5/6),3Ns,4Fs [Hoyer et al., 2012]	3Del(0.73–2.72),2Ns,1Fs [Santen et al., 2012]	Del(1.19Mb) [Michelson et al., 2012]
Growth						
Prenatal growth (birth weight/length)#	−2.3/−3.7	3/4		0/9	0/6	−
Postnatal growth (weight/height)†	−2.3/−6.1	1/3	5/7	3/9	3/6	−
Psychomotor						
Developmental delay/mental retardation‡	Severe	4/4(2Moderate, 2Severe)	8/8	5Moderate,4Severe	2Moderate, 4Severe	Moderate
Speech delay	NW	4/4(3NW, 1SW)	8/8(3NW,5SW)	9/9(2NW, 2SW, 3Se)	6/6(2NW, 3Severe, 1Moderate)	NW
Seizures	+	1FC	3/6	3/9		+
Hypotonia	−	2	7/8	7/9		
Autistic features/behavioral abnormalities	HA		5/7	1	1	
Microcephaly (≤2 SD) (SD score)	+	4/4	1/6	2/9	0/6	−
Brain anomaly	−?	2/3(ACC + Colp)	4/5(1ACC, 3HCC, 1HCV)	0/6	4/4(3ACC, 1HCC, 2Colp)	HCC
Craniofacial						
Sparse hair	+		1	1	1	
Thick eyebrows	+		1		6/6	
Thick/long/prominet eyelashes	−					
Ptosis	−	1				
Abnormal ears	+	2	1	7/9		
Nasal bridge	Narrow	3Broad	3Broad			Broad
Thick, anteverted alae nasi						+
Wide mouth	−		2	1		
Philtrum	Long	2Long	2Long	1Long		
Upper lip vermilion feature		2Thin	3Thin,1Thick	6/9(6Thin)		Thin
Thick lower lip vermilion	−	2Thick	3			
Palatal abnormality	H	2H		2/9(2H)		
Skeletal-limb						
Hypoplastic/absent fifth finger/toe	Fi/T				2/6(2Fi)	
Hypoplastic/absent nail (fifth finger/toe)	+		1T	1T	2/6(2Fi)	
Hypoplastic/absent nail (other fingers/toes)				1?	1/6	
Prominent interphalangeal joints	+					
Prominent distal phalanges	−	1				
Scoliosis/spinal abnormalities	+		1	1		
Joint laxity	−		3/4		3/5	
Others						
Hirsutism	+		3/4	2		3/6
Congenital heart defects	MR	1AtSD	1AtSD	2/9(1AtSD)	1AtSD	
Genitourinary defects	−	1PSW	1Cr, 1DoUr, 2RL	2Cr, 1MU	1RL, 1DoUr	
Gastrointestinal abnormalities	GU, GER		1Co, 1AA	1AA		
Inguinal (I)/umbilical (U) hernia	−					
Sucking difficulty	−	3	4			+
Feeding difficulty	−		5	+		
Frequent vomiting						
Hearing impairment	−	4	1/2	1/9	+	
Visual impairment	−	1St, 1Am	2Hy, 2My, 2St,1 Cat, 1Ny		3St/9,4My	2St
Recurrent infections	+	1	1			

+, present; −, absent; blank, data not available; §, Tsurusaki et al. [2013]; #, SD score; †, SD acore; ‡, at latest assessment; AA, anal atresia; ACC, agenesis of corpus callosum; Am, amblyopia; AtSD, atrial septal defect; Cat, cataract; Co, constipation; Colp, colpocephaly; Cr, cryptorchidism; Del, deletion; Dup, duplication; DoUr, double ureter; F, female; Fi, finger; Fs, frameshift mutation; GER, gastrointestinal reflux; GU, gastric ulcer; H, high palate; HA, hyperactivity; HCC, hypoplastic corpus callosum; HCV, hypoplastic cerebellar vermis; Hy, hypermetropia; M, male; MR, mitral regurgitation; MU, megaureter; My, myopia; Ns, nonsense mutation; NW, no words; Ny, nystagmus; PSW, penoscrotal webbing; RL, renal lithiasis; Se, sentences; St, strabismus; SW, several words; T, toe; Tra, translocation; fractions in the previous publications show patient numbers "feature-positive/data available"
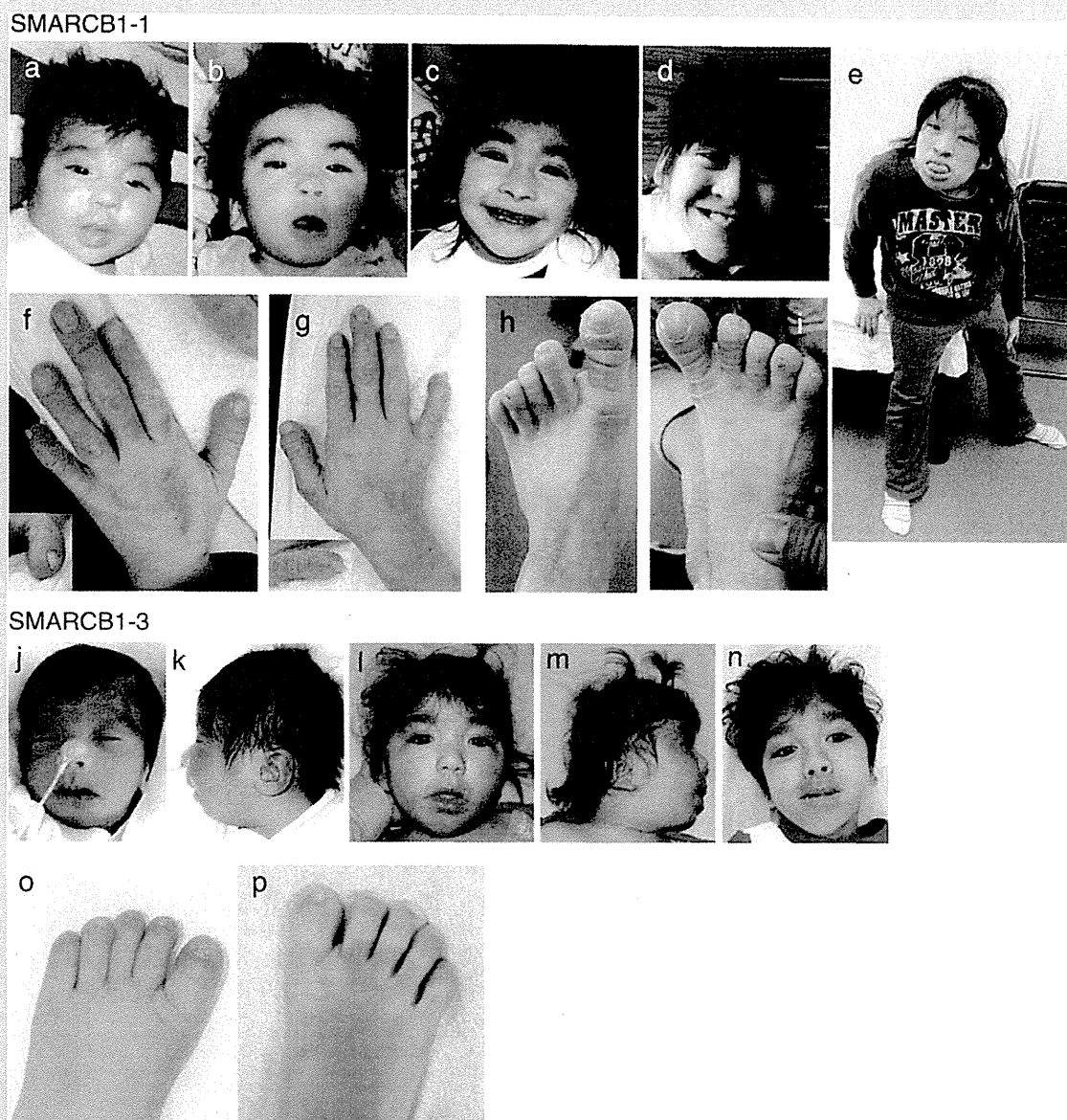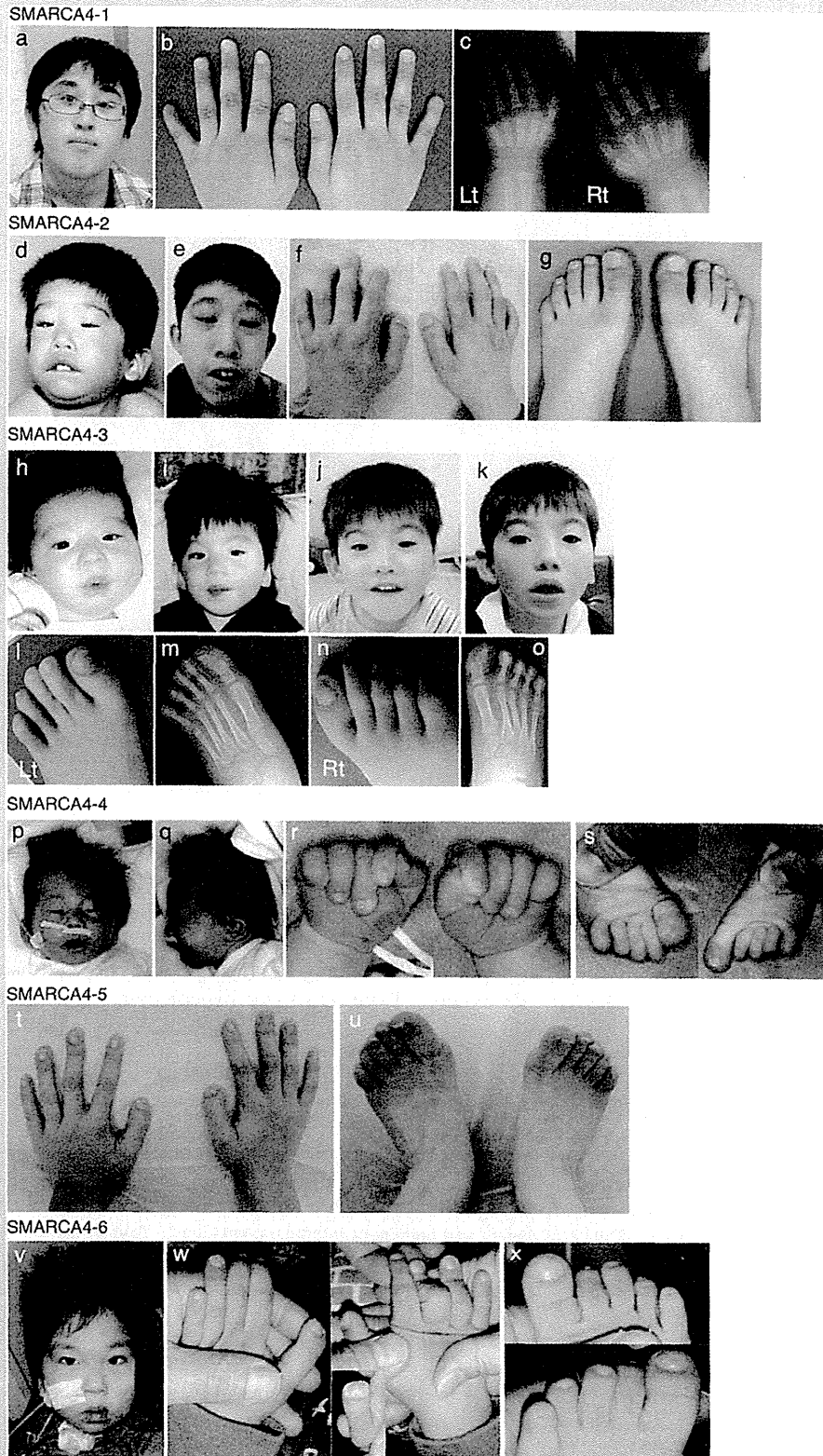
FIG. 1. Clinical photographs of patients with an *SMARCB1* mutation. SMARCB1-1: Craniofacial features at age 2 months (a), 1 year (b), 6 years (c), and 18 years (d). Note a round face with thick and arched eyebrows, a short nose with a bulbous tip and anteverted nostrils, a long philtrum, a small mouth, and micro-retrognathia in the early childhood. Later, note a broad nasal bridge without anteverted nostrils, a broad philtrum, a large tongue, and a protruding jaw. Poor posture due to severe scoliosis (e) as well as hypoplastic fingers (f, g) and toes (h, i) with nail hypoplasia, prominent interphalangeal joints, and prominent distal phalanges are noted at age 21 years. SMARCB1-3: Craniofacial features in the neonatal period (j, k), at age 2 years (l, m), and 7 years (n). Note a round face with thick and arched eyebrows, a short nose with anteverted nostrils, a long philtrum, a small mouth, and micro-retrognathia in early childhood. Later, note a broad nasal bridge and a protruding jaw. Feet at age 5 years (o, p). Note hypoplasia of the bilateral fifth toes and hypoplasia of all toenails. [Figure I originally published in Tsurusaki et al. [2012], in *Nature Genetics*.]

156 cm (−2.6 SD), and an OFC of 51.2 cm (−3.8 SD). He suffers from constipation, nocturnal enuresis, and unstable body temperature. He understands simple commands and speaks several words. He is friendly but also hyperactive and impulsive.

SMARCA4-3 (Subject 5 [Tsurusaki et al., 2012]; Fig. 2h–o): Increased nuchal translucency thickness was shown by fetal ultra-sonography. He was born at 41 weeks of gestation. His birth weight was 2,756 g (−1.2 SD), length was 48 cm (−0.9 SD), and OFC was 32.0 cm (−0.9 SD). He was gavage-fed due to sucking/feeding difficulties until 7 months of age. He had right cryptorchidism which was corrected surgically at age 1 year. He showed hypotonia and motor development was delayed: he raised his head at age
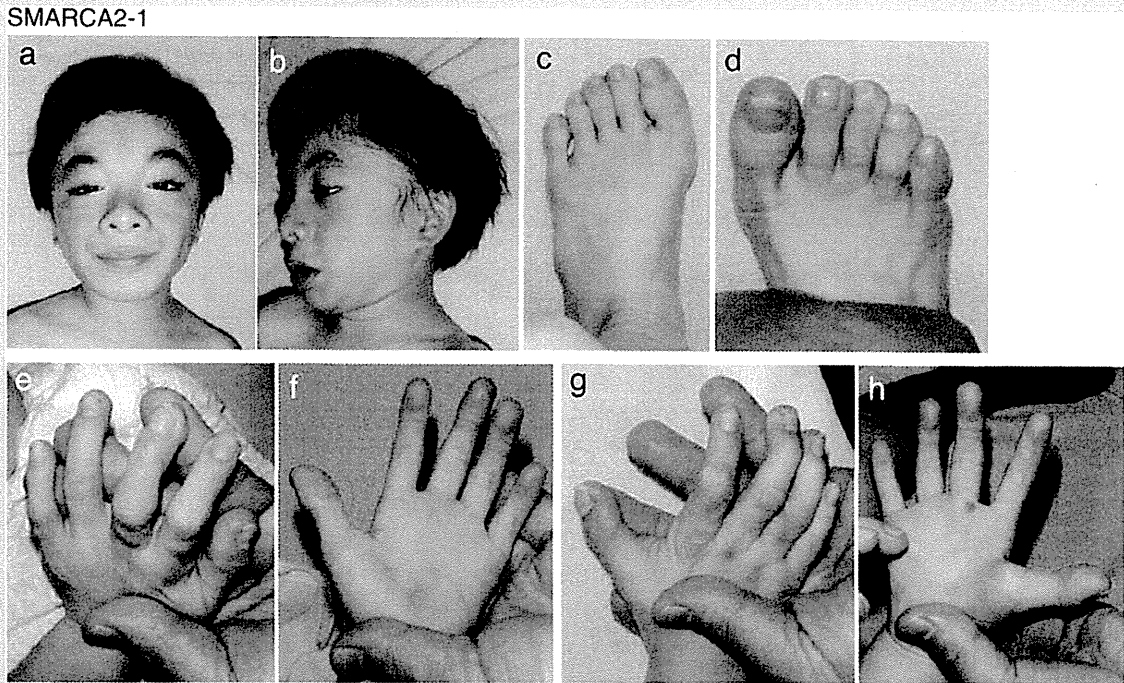
FIG. 3. Clinical photographs of a patient (SMARCA2-1) with an *SMARCA2* mutation. Craniofacial features (a, b), feet (c, d), and hands (e–h) at age 6 6/12 years. Note sparse hair, thick, and arched eyebrows, a broad nasal bridge with anteverted nostrils, a broad philtrum, a wide mouth, and thick upper and lower lip vermilions; and prominent interphalangeal joints and prominent distal phalanges of all fingers and toes without nail hypoplasia. (Figure a, d originally published in Tsurusaki et al. [2012], in *Nature Genetics*.)

7 months, sat alone at 1 3/12 years, and walked independently at 6 years. His developmental quotient (DQ) was 17 at age 5 9/12 years. MRI of the brain at age 6 11/12 years showed hypoplasia of the upper cerebellar vermis and mild hypoplasia of the corpus callosum. At age 9 10/12 years, he weighs 22.1 kg (−1.5 SD), has a height of 114 cm (−3.2 SD), and an OFC of 48 cm (−3.6 SD). He suffers from unstable body temperature, facial flushing, aerophagia, and intractable constipation. His skeletal problems have included bilateral genu recurvatum with an episode of patella dislocation, bilateral pes valgus, subluxation in the left hip, and Perthes disease-like changes in the right hip. He has autism spectrum disorder with hypersensitivity, hyperactivity, self-injurious behaviors, obsession,

FIG. 2. Clinical photographs of patients with *SMARCA4* mutations. SMARCA4-1: Craniofacial features (a) and hands (b) at age 18 years and hand radiographs (c) at age 5 months. Note a slender face with thick eyebrows, an everted upper lip vermilion, a protruding jaw; hypoplasia of bilateral distal phalanges of the first and fifth fingers, and bilateral hypoplastic fifth fingernails. SMARCA4-2: Craniofacial features at age 2 years (d) and 20 years (e). Note hypertrichosis, a narrow forehead, blephalophimosis that was corrected surgically, thick and slightly arched eyebrows, long eyelashes, a short and low nose with anteverted nostrils, a short philtrum, an everted upper lip vermilion, and low-set ears in the early childhood; and later a slender face with a long nose and a thick lower lip vermilion. Hands at age 20 years (f). Note hypoplasia of the distal phalanges and nails of bilateral fingers and prominent interphalangeal joints. Feet at age 20 years (g). Note prominent distal phalanges of bilateral first toes and hypoplasia of bilateral fifth toes as well as aplasia of bilateral fifth toenails. SMARCA4-3: Craniofacial features at age 4 months (h), 2 2/12 years (i), 5 1/12 months (j), and 9 10/12 years (k). Note arched eyebrows growing thicker, left ptosis, prominent ears, an everted upper lip vermilion, and a thick lower lip vermilion. Feet at age 9 10/12 years (l–o). Note prominent distal phalanges of bilateral first toes, hypoplasia of bilateral second-to-fifth toes, hypoplasia of bilateral fourth toenails, and aplasia of bilateral fifth toenails. SMARCA4-4: Craniofacial features (p, q), hands (r), and feet (s) in the neonatal period. Note a round face with thin and arched eyebrows, blephalophimosis, low-set ears, a thin upper lip vermilion, and micro-retrognathia; hypoplasia of bilateral fifth fingers and toes, aplasia of bilateral fifth fingernails and toenails, and hypoplasia of bilateral second-to-fifth fingernails and toenails. SMARCA4-5: Hands (t) and feet (u) at age 8 years. Note hypoplasia of bilateral fifth fingers and toes, hypoplasia of bilateral first and fifth fingernails and second-to-fifth toenails, prominent distal phalanges of bilateral first toes, and prominent interphalangeal joints. SMARCA4-6: Craniofacial features (v), hands (w), and feet (x) at age 3 7/12 years. Note thick and arched eyebrows and small mouths, hypoplasia of bilateral fifth fingers and toes, bilateral all fingernails and bilateral first-to-fourth toenails, aplasia of bilateral fifth toenails, and prominent distal phalanges of bilateral first toes. (Figure k, n, p, r originally published in Tsurusaki et al. [2012], in *Nature Genetics*.)
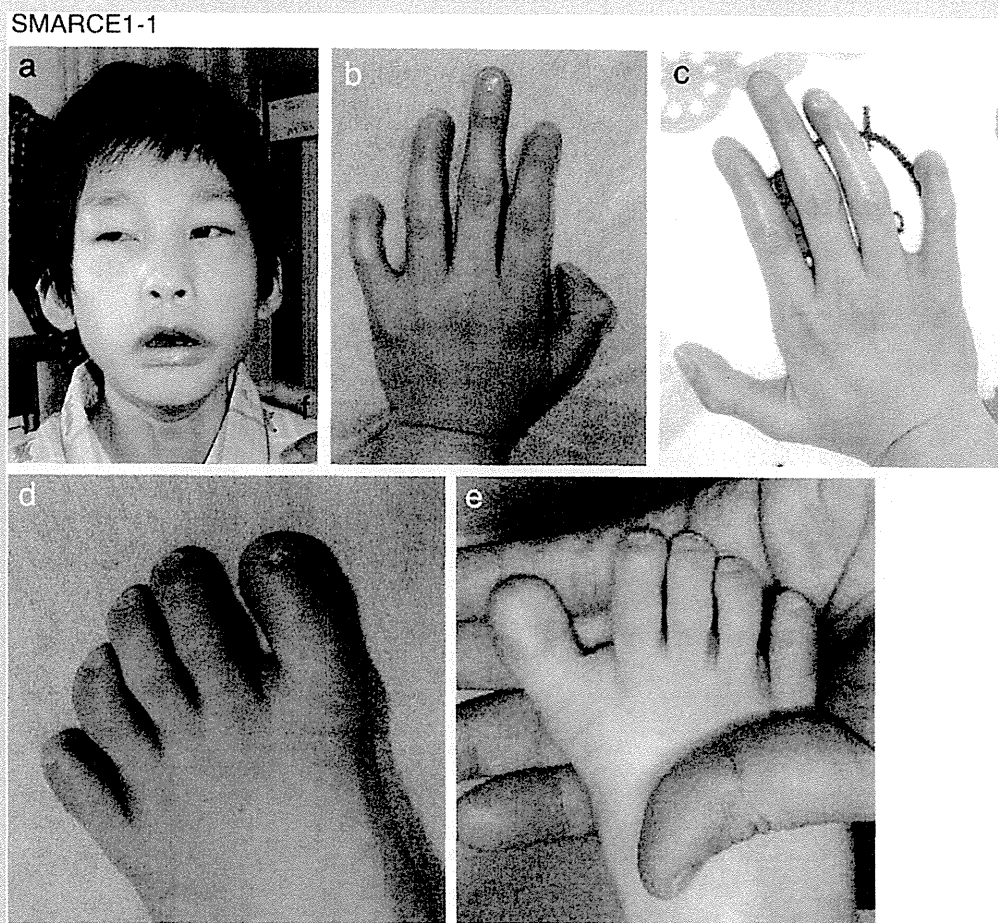
SMARCE1-1

FIG. 4. Clinical photographs of a patient (SMARCE1-1) with an *SMARCE1* mutation. Craniofacial features (a), hands (b, c), and feet (d, e) at age 14 years. Note a slender face with slightly thick eyebrows, a narrow nasal bridge with anteverted nostrils, low-set prominent ears, a long philtrum, an everted upper lip vermilion, and a thick lower lip vermilion; hypoplasia of bilateral second and fifth fingers and bilateral first toes, hypoplasia of all finger/toenails, and aplasia of bilateral fifth fingernails and the right first toenail. (Figure e originally published in Tsurusaki et al. [2012], in *Nature Genetics*.]

and severely retarded language development (developmental age on speech, 6–7 months; comprehension, 10 months).

SMARCA4-4 (Subject 16 [Tsurusaki et al., 2012]; Fig. 2p–s): He was born at 41 weeks of gestation. His birth weight was 2,502 g (−1.7 SD), length was 47 cm (−1.6 SD), and OFC was 33 cm (−0.6 SD). He showed respiratory insufficiency with apnea because of laryngomalacia, which required oxygen supplementation for 15 days. He had sucking/feeding difficulties and was gavage-fed until 7 months of age. He had a small VSD and a small patent ductus arteriosus (PDA): both closed spontaneously. At age 1 4/12 years, he had surgical correction for bilateral inguinal herniae. At age 1 9/12 years, he underwent emergency surgery for a perforated duodenal ulcer. Motor development was severely delayed: he raised his head at age 7–8 months, stood with support at 1 10/12 years, and walked independently at 6 years. At age 11 years, he weighs 22.9 kg (−1.8 SD), has a height of 122.5 cm (−3.1 SD), and an OFC of 49.3 cm (−2.9 SD). He has mild pulmonary regurgitation and

optic disk coloboma with reduced visual acuity (0.3). He experiences constipation and rhinitis, and has difficulty in opening his mouth. He is friendly and understands language, but speaks no words and communicates to others with gestures. Autistic behaviors have not been reported.

SMARCA4-5 (Subject 25 [Tsurusaki et al., 2012]; Fig. 2t, u): She was born at 40 weeks of gestation. Her birth weight was 2,820 g (−1.0 SD), length was 46 cm (−1.9 SD), and OFC was 33.5 cm (+0.1 SD). She suffered from recurrent infections, sucking/feeding difficulties, constipation, visual impairment, and hearing loss in the left ear. She showed hypotonia and motor development was severely delayed: she raised her head at age 8 months, sat alone at 1.5 year, and walked independently at 2 8/12 years. The DQ at age 8 years was 38. MRI of the brain showed hypoplasia of the corpus callosum. At age 16 years, she weighs 39 kg (−1.9 SD), has a height of 148 cm (−1.9 SD), and an OFC of 52 cm (−2.3 SD). She suffers from constipation and scoliosis (which is treated with a brace). She makes
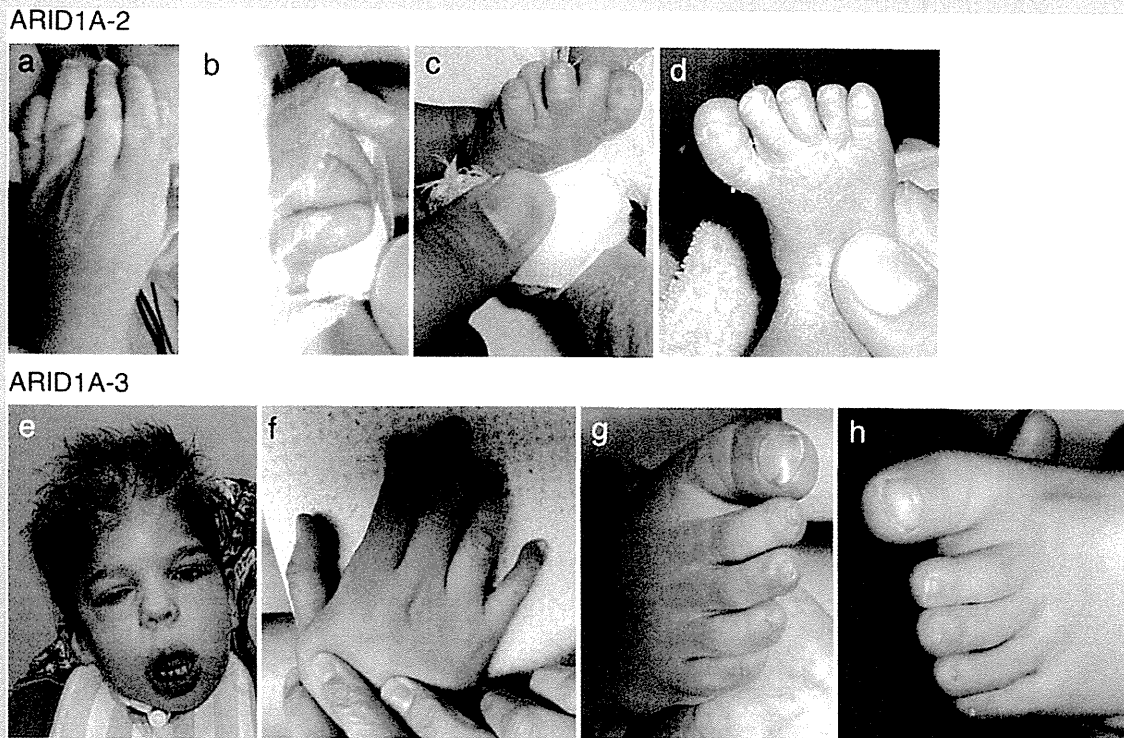
ARID1A-2

ARID1A-3

FIG. 5. Clinical photographs of patients with *ARID1A* mutations. ARID1A-2: Hands (a, b) and feet (c, d) in the neonatal period. Note hypoplasia of the left fifth finger and the left fifth toe, severe hypoplasia of bilateral fifth fingernails and the left fifth toenail, and mild hypoplasia of the other finger/toenails. ARID1A-3: Craniofacial features (e), the right hand (f), and feet (g, h) at age 10 years. Note thick eyebrows, downslanting palpebral fissures, hemifacial microsomia, a long philtrum, thick upper and lower lip vermilions, and a large mouth; hypoplasia of bilateral fifth toes, hypoplasia of all finger/toenails, and prominent distal phalanges of fingers and toes. (Figure e originally published in Tsurusaki et al. [2012], in *Nature Genetics*.)

simple conversations and reads "hiragana" (Japanese cursive characters). She needs a lot of help in her daily life. She shows repetitive behavior (moving her hands) but other autistic behaviors have not been reported.

SMARCA4-6 (Subject 17 [Tsurusaki et al., 2012]; Fig. 2v–x): He was born at 41 weeks of gestation. His birth weight was 2,704 g (−1.1 SD), length was 46 cm (−2.3 SD), and OFC was 32 cm (−1.3 SD). He was admitted to hospital for respiratory insufficiency with cyanosis and multiple congenital anomalies (cleft palate, broad thumbs, omphalocele, bilateral cryptorchidism). He had surgical correction of omphalocele at age 1 day. He had complex heart defects, including mitral atresia, pulmonary atresia, a single right ventricle, an atrial septal defect (ASD) and a PDA, and received intravenous administration of prostaglandin E1. He underwent a right Blalock–Taussig shunt at age 1 month, surgical enlargement of the ASD at age 1 1/12 year, a left Blalock–Taussig shunt at age 2 years, a Glenn procedure at age 3 3/12 years, and a Fontan procedure at age 4 6/12 years. He had tracheostomy for hypoxemia caused by bronchomalacia at age 1 8/12 years. He suffered from recurrent infections. His motor development was severely delayed: he raised his head at age 7 months and rolled over at 1 year. MRI of

the brain at age 1 year showed hypoplasia of the corpus callosum. At age 4 9/12 years, he weighs 11.1 kg (−3.0 SD), has a height of 90.6 cm (−3.4 SD), and an OFC of 46.6 cm (−2.7 SD). He underwent laparoscopic fundoplication and gastrostomy for GER. He is unable to sit alone.

SMARCA4-7: She was born at 41 weeks of gestation. Her birth weight was 2,230 g (−2.6 SD), length was 45 cm (−2.7 SD), and OFC was 28 cm (−3.9 SD). She had sucking/feeding difficulties and vomited frequently due to GER. She suffered from recurrent infections. Inguinal and umbilical herniae were corrected surgically. She showed hypotonia and motor development was moderately delayed: she walked independently at age 2 6/12 years. At age 8 years, she weighs 17.4 kg (−1.9 SD), has a height of 115.8 cm (−1.8 SD), and an OFC of 47.5 cm (−3.0 SD). She speaks three-word sentences but cannot read "hiragana." She is hyperactive.
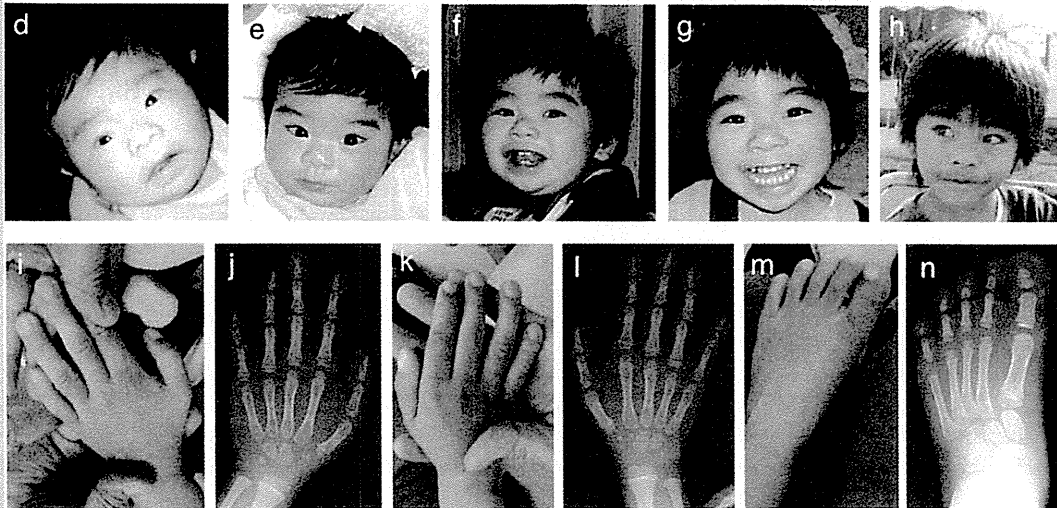
SMARCA2 Mutation

SMARCA2-1 (Subject 19 [Tsurusaki et al., 2012]; Fig. 3a–h): He was born at 38 weeks of gestation. His birth weight was 1,774 g (−2.6 SD), length was 42 cm (−3.1 SD), and OFC was 32 cm (−0.5 SD).
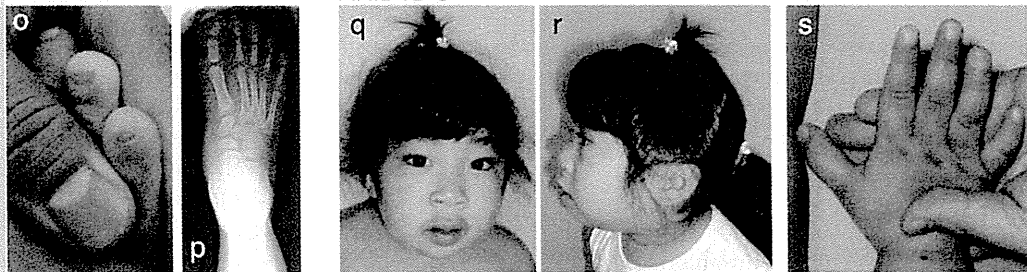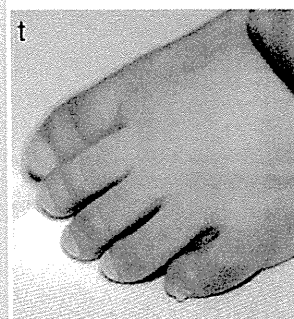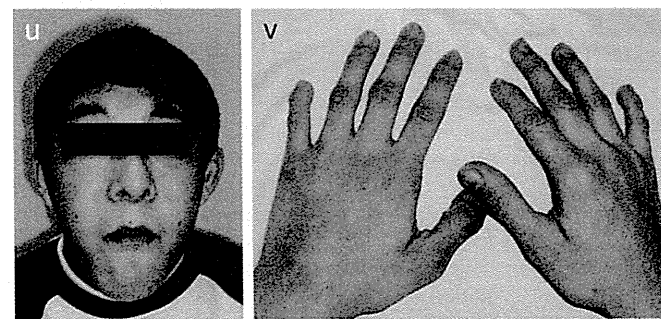
ARID1B-1
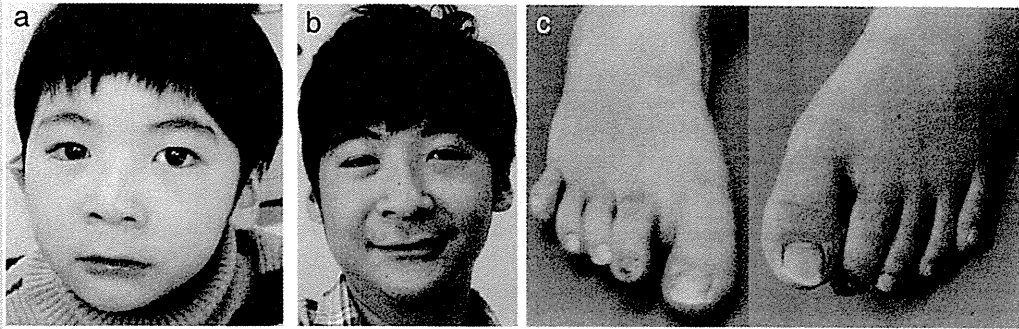
ARID1B-2

ARID1B-3

ARID1B-4

ARID1B-5

He had sucking/feeding difficulties. He underwent surgical correction for a left inguinal hernia and cryptorchidism as well as an umbilical hernia at age 4 months; for recurrence of the umbilical hernia at age 2 3/12 years; and for hypospadias at age 3 1/12 years. He developed a complex febrile convulsion at age 1 8/12 years and an afebrile seizure at age 7 years. At age 6 years, renal biopsy for gross hematuria showed immunoglobulin (Ig)A nephropathy that was treated through corticosteroids and tonsillectomy. A radiograph at age 7 years showed scoliosis with a Cobb angle of 38° at Th6–L1 that required a brace. Ventilation tubes were placed for chronic exudative otitis media. He showed hypotonia and motor development was delayed: he raised his head at 4 months, sat alone at 9 months, and walked independently at 3 8/12 years. At age 8 7/12 years, he weighs 16.9 kg (−2.1 SD), has a height of 107.8 cm (−3.8 SD), and an OFC of 47.8 cm (−3.3 SD). He vomits readily if he is in poor physical condition such as infection. He speaks no words. Autistic behaviors have not been reported.

SMARCE1 Mutation

SMARCE1-1 (Subject 24 [Tsurusaki et al., 2012]; Fig. 4a–e): She was born at 37 weeks of gestation. Her birth weight was 1,642 g (−2.3 SD). Mechanical ventilation was required for the first 7 months. She was gavage-fed and underwent gastrostomy at age 10 years. Tricuspid regurgitation, mitral regurgitation, and aortic stenosis were detected and have been treated with diuretics. Seizures occurred in her infancy, and valproic acid and carbamazepine were administered. Cleft palate and progressive scoliosis were corrected surgically. She had hearing impairment that was profound in the right ear and which was moderate (threshold, 50 dB) in the left ear. She suffered from recurrent infections. At age 14 years, she weighs 15.3 kg (−4.4 SD), has a height of 121 cm (−6.8 SD), and an OFC of 45 cm (−6.7 SD). She has to be supported to stand up, but can walk and trot independently (albeit in an ataxic manner) from a standing position. She speaks no words. She can assess her situation simply and can operate a DVD player.

ARID1A Mutations

ARID1A-1 (Subject 3 [Tsurusaki et al., 2012]): Intrauterine growth retardation, enlargement of the third ventricle and posterior fossa,

and a single umbilical artery were detected through fetal ultrasonography. He was born at 39 5/7 weeks of gestation. His birth weight was 2,675 g (−1.0 SD) and length was 47 cm (−1.4 SD). He had coarctation of the aorta, an ASD, and a VSD; the ASD was closed surgically at age 3 months. Congenital pyloric stenosis was corrected surgically at age 12 days, but GER developed. He suffered from sucking/feeding difficulties and frequent infections. MRI of the brain showed agenesis of the corpus callosum and a posterior fossa arachnoid cyst. At age 2 1/12 years, he weighed 3,969 g (−6.2 SD), had a height of 59.6 cm (−8.9 SD), and an OFC of 41.7 cm (−3.7 SD). Hepatoblastoma, noticed at age 1 10/12 years, progressed to death at 2 3/12 years.

ARID1A-2 (Subject 6 [Tsurusaki et al., 2012]; Fig. 5a–d): He was born at 37 weeks of gestation. His birth weight was 2,546 g (−0.6 SD), length was 45 cm (−1.1 SD), and OFC was 36.4 cm (+2.2 SD). He was admitted to the Neonatal Intensive Care Unit (NICU) due to asphyxia, multiple malformations (choanal atresia, cleft palate, hypospadias, and bilateral cryptorchidism) as well as profound hypotonia. Mechanical ventilation was started for respiratory weakness, and tracheostomy carried out at age 9 months. Cardiovascular complications included a VSD, coarctation of the aorta, and stenosis of the aortic valve, which were corrected surgically at age 20 days. A colostomy was placed at age 6 days for anal atresia with retrourethral fistula. Agenesis of the corpus callosum, cerebellar hypoplasia, and a Dandy–Walker malformation were detected. Motor development was severely delayed: he was bedridden with no head control. He suffered from recurrent urinary tract infections. At age 1 year, atrioventricular block occurred with a heart rate of ≈40–50/min, and he died 1 month later. He weighed 3,940 g (−5.5 SD), had a height of 55.0 cm (−8.2 SD), and an OFC of 41.9 cm (−3.1 SD).

ARID1A-3 (Subject 8 [Tsurusaki et al., 2012]; Fig. 5e–h): He is a Caucasian male, born at term after an uncomplicated pregnancy and delivery. His birth weight was 3,350 g. He showed respiratory distress due to laryngomalacia and was admitted to the NICU. MRI of the brain in the neonatal period showed agenesis of the corpus callosum. At age 6 months, he had surgery for an inguinal hernia and intestinal obstruction, and a gastrostomy was undertaken due to sucking/feeding difficulties and GER. A large ASD closed spontaneously before the age of 3 years. Bilateral strabismus repair was carried out at age 1 year. He had mild hearing impairment in the

FIG. 6. Clinical photographs of patients with *ARID1B* mutations. ARID1B-1: Craniofacial features at age 5 years (a) and 13 years (b). Note thick and arched eyebrows, a broad nasal bridge, a broad philtrum, and a thin upper lip vermilion. Feet at age 13 years (c). Note hypoplasia of the right fifth toe, hypoplasia of the right fifth toenail and the left fourth and fifth toenails, and prominent distal phalanges of bilateral first toes. ARID1B-2: Craniofacial features in the neonatal period (d), at age 5 months (e), 1 3/12 years (f), 3 4/12 years (g), and 6 8/12 years (h). Note thick eyebrows, a broad nasal bridge with anteverted nostrils, a broad philtrum, a thin upper lip vermilion, and change in facial shape from round to slender. Hands (i, left; k, right) with the radiographs (j, left; l, right) and feet (m, left; o, right) with the radiographs (n, left; p, right) at age 6 9/12 years. Note hypoplasia of bilateral fifth toes and toenails and prominent distal joints in bilateral second-to-fifth fingers and the left first toes. ARID1B-3: Craniofacial features (q, r) and the right hand (s) at age 4 5/12 years. Note a round face with thick eyebrows, a broad nasal bridge, a long philtrum, a thick lower lip vermilion, a small mouth, and micro-retrognathia; and hypoplasia of the fifth finger with mild hypoplasia of the fifth fingernail. ARID1B-4: The left foot (t) at age 6 months. Note hypoplasia of the fifth toe and the toenail. ARID1B-5: Craniofacial features (u) and hands (v) at age 19 years. Note a slender face with thick and arched eyebrows, a narrow nasal bridge, a long philtrum, and a protruding jaw; hypoplasia of bilateral fifth fingers and fingernails, prominent interphalangeal joints of all fingers, and prominent distal phalanges of bilateral first and fifth fingers. (Figure b and o originally published in Tsurusaki et al. [2012], in *Nature Genetics*.)