CLINICAL REPORT

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Pure Duplication of 19p13.3

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Chromosomal abnormalities involving 19p13.3 have rarely been described in the published literature. Here, we report on a girl with a pure terminal duplication of 6.1 Mb on 19p13.3, caused by an unbalanced translocation der(19)t(10;19)(qter;p13.3)dn. Her phenotype included severe psychomotor developmental delay, skeletal malformations, and a distinctive facial appearance, similar to that of a patient previously reported by Lybaek et al. [Lybaek et al. (2009); Eur J Hum Genet 17:904–910]. These results suggest that a duplication of >3 Mb at the terminus of 19p13.3 might represent a distinct chromosomal syndrome.

Key words: 19p13.3 duplication; array CGH; developmental delay; subtelomere

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INTRODUCTION

Chromosome 19 is more gene-dense than any other human chromosome. Non-mosaic 19p trisomy is a rare chromosomal aberration, of which only 9 occurrences have been reported to date [Byrne et al., 1980; Salbert et al., 1992; Stratton et al., 1995; Andries et al., 2002; Puvabanditsin et al., 2009; Lybaek et al., 2009; Descartes et al., 2011; Siggberg et al., 2011; Lehman et al., 2012]. More specifically, pure and non-mosaic trisomy of 19p has been reported in only five of these patients [Stratton et al., 1995; Andries et al., 2002; Lybaek et al., 2009; Siggberg et al., 2011; Lehman et al., 2012].

Here, we report on a 3-year-old girl with pure terminal duplication of 19p13.3, confirmed using FISH and array CGH. She had multiple malformations, including a complex congenital heart defect, a distinctive facial appearance, and severe developmental delay. Taken together, our findings, along with a review of the literature, allow clarification of a more precise and comprehensive phenotype–genotype correlation for pure 19p duplication.

CLINICAL REPORT

The proposita is the first child of healthy unrelated parents with unremarkable family history. At the time of delivery, the mother was 36 years old, and the father was 27 years old. The pregnancy was complicated by intrauterine growth retardation first noted at 27 weeks. The infant was delivered at 35 weeks of gestation by cesarean due to fetal distress. Her birth weight was 1,216 g; length, 36.5 cm; and occipitofrontal circumference (OFC), 28.0 cm. Her Apgar scores were 4 at 1 min, and 9 at 5 min. Because of her very low birth weight and respiratory failure, she was admitted to a neonatal intensive care unit. Initial physical examination showed a distinctive facial appearance with micrognathia, low-set ears, and a prominent occiput. An echocardiogram revealed a complete atrioventricular septal defect of Rastelli A type, severe pulmonary hypertension, and mitral valve dysplasia.

At the age of 8 months, catheter examination demonstrated that an operative procedure was not indicated for her heart defects; conservative treatment with beraprost sodium and bosentan hydrate, in addition to oxygen supplementation, was adopted for heart failure and severe pulmonary hypertension. From the age of 1 year and 8 months, sildenafil citrate was also added to her treatment. At this age, she had marked cardiac failure and had experienced several episodes of recurrent respiratory infection.

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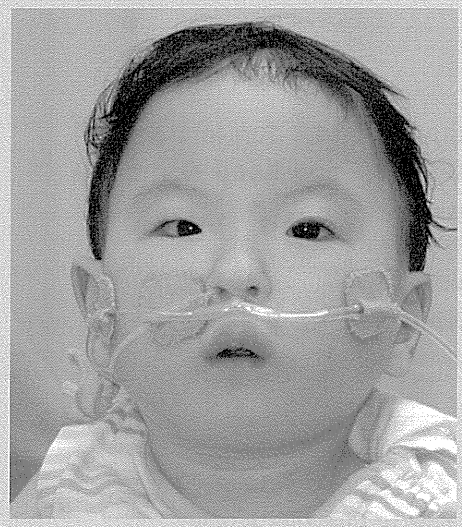

FIG. 1. Patient at the age of 3 years, showing strabismus, short palpebral fissures, hypoplastic nasal alae, low-set ears, and microstomia.

On examination at the age of 3 years, her weight was 7,680 g (−3.4 SD); height, 75 cm (−5.0 SD); and OFC, 42 cm (−4.0 SD) (Fig. 1). Her facial appearance showed strabismus, short and downslanting palpebral fissures, microcephaly, hypoplastic nasal alae, sparse scalp hair, and eyebrows, low-set ears, a short philtrum, protruding upper lip, and microstomia with micrognathia. Orthopedic examination showed kyphoscoliosis and dislocation of bilateral hip joints. Her development was severely delayed. She could roll over and required gavage feeding. Her heart failure progressed, and died at age 4 years. Postmortem examination revealed an ectopic left kidney in front of the vertebrae.

MATERIALS AND METHODS

Written informed consent was obtained from the parents of the patient, and the study was performed in accordance with the Kanagawa Children's Medical Center Review Board and Ethics Committee.

An initial FISH analysis for patients with developmental delay/intellectual disability (DD/ID) and/or multiple congenital anomalies (MCA) was carried out using subtelomeric probes (Vysis, Downers Grove, IL) according to the standard protocol. Further FISH analysis for determining the breakpoint on 19p13.3 was carried out using bacterial artificial chromosome (BAC) clones that had been selected from the May 2004 (NCBI35/hg17). Human assembly of the UCSC Genome Browser (http://genome.ucsc.edu/). A centromeric probe specific for chromosome 10 was used to confirm chromosome 10. The BAC clones were labeled by nick translation according to the manufacturer's instructions (Vysis, Downers Grove, IL). Hybridization, post-hybridization washing, and counterstaining were performed according to standard procedures. Slides were analyzed using a completely motorized epifluorescence microscope (Leica DMRXA2; Leica Microsystems Imaging Solutions, Cambridge, UK) equipped with a CCD camera. Both the camera and microscope were controlled with Leica CW4000 M-FISH software [Yamamoto et al., 2009].

Array comparative genomic hybridization (array-CGH) was performed using the Agilent SurePrint G3 Human CGH Microarray Kit 8 × 60K (Agilent Technologies, Inc., Santa Clara, CA). The total genomic DNA of the patient was prepared using standard techniques. The results were analyzed using Agilent Genomic Workbench software. Only experiments having a derivative log ratio (DLR) spread value <0.30 were considered.

RESULTS

The complete subtelomere probe set analysis detected an additional signal for 19pter on the terminal of the long arm in group C chromosomes in the patient. Based on the results of the G-banding patterns and FISH with a centromeric probe, the derivative chromosome was determined to be chromosome 10 (Fig. 2a,b). However, the 10qter probe signal was retained in the derivative chromosome (data not shown). To characterize the size of the deletion, we further applied FISH analysis using the BAC clones that mapped to the region. This revealed that the breakpoint was 6.1 Mb from 19pter (Table I). Subsequent array-CGH analysis revealed a 19p13.3 duplication of approximately 6.1 Mb (chr19: 327,273–6,106,229), which was consistent with the FISH results (Fig. 2c). No other genomic imbalances were identified on the array analysis. FISH analysis with relevant BAC clones indicated that the duplication was absent in both parents, and therefore had occurred de novo.

DISCUSSION

Reports of abnormalities of the short arm chromosome 19 are rare; to date, only nine patients with non-mosaic duplication of 19p have been reported. Of these, four involved translocation of other chromosomes [Byrne et al., 1980; Salbert et al., 1992; Puvabanditsin et al., 2009; Descartes et al., 2011], and only five patients had a pure partial duplication of 19p [Stratton et al., 1995; Andries et al., 2002; Lybaek et al., 2009; Siggberg et al., 2011; Lehman et al., 2012] (Fig. 3., Table II). This report is, to our knowledge, only the second report of a pure terminal duplication of 19p13.3.

Array-CGH and FISH analysis refined the breakpoint at 6.1 Mb from 19pter. Three patients harboring a duplication of more than 1 Mb at 19p13.3 have been recorded on the DECIPHER database (https://decipher.sanger.ac.uk/), but no individual with a duplication of more than 3 Mb is recorded therein. Fourteen patients having a duplication of a fragment of 19p13.3 have been reported in the database of International Standards for Cytogenetic Arrays Consortium (ISCA). The phenotypical manifestations of these patients consist of multiple congenital abnormalities and seizures. However, the detailed phenotypic features of the patients were not available. Although the phenotype deriving from duplication of a limited region of 19pter is not always recognizable [Andries

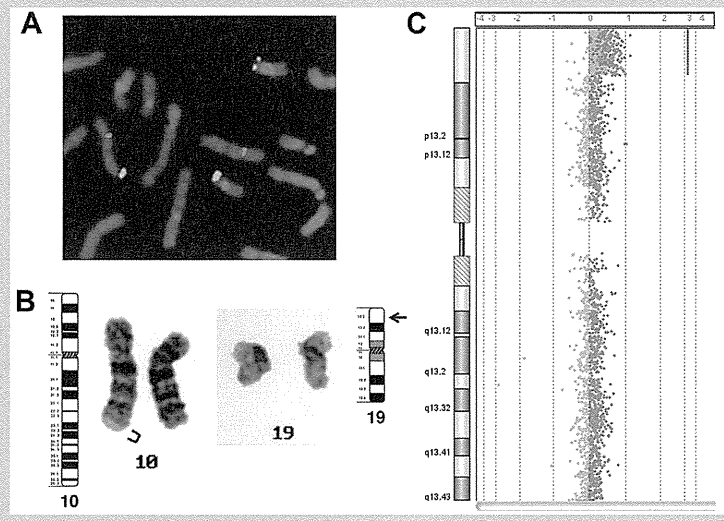

FIG. 2. FISH and array-CGH characterization of 19p13.3 terminal duplication. A: FISH image showing an additional signal at 10qter. BAC probe RP11-43H17 from the duplicated region of 19p13.3 is labeled in green, and chromosome 10 centromeric probe (Vysis, CEP10) is labeled in red, as a control. B: G-banded metaphase chromosomes, showing der(10)t(10;19)(qter;p13.3). C: Array-CGH showing duplication of 19p13.3. The region extends to position 6,106,229 according to UCSC human genome assembly build 19.

et al., 2002], the present case presented with severe psychomotor disability, no verbal language use, a distinctive facial appearance, and skeletal features including small hands and feet and bilateral hip dysplasia. These phenotypic features, especially the characteristic facial appearance, were also shared by the patient described by Lybaek et al. [2009]. The patient had a small mouth, short philtrum, full cheek, short palpebral fissures, and the extreme precocious puberty before the age of 5 months. They demonstrated that only about 25% of the duplicated 215 genes presented the overall expression pattern by more than 1.3-fold, and suggested no genes might explain the precocious puberty characterized in their patient. However, our present patient had no symptom of early puberty observed by the age of 4 years.

TABLE I. FISH Results Around the Breakpoint of the Translocation

BAC clones	Position from 19pter[a]	FISH results
RP11-1051P16	3,421,215–3,617,048	×3
RP11-43H17	4,318,718–4,491,568	×3
RP11-348B12	4,960,407–5,144,570	×3
RP11-294F21	5,854,144–6,041,711	×3
RP11-576B17	6,172,183–6,249,454	×3
RP11-114A7	6,199,888–6,359,433	×2
RP11-30F17	6,351,112–6,519,252	×2
RP11-459P1	6,396,557–6,544,479	×2
RP11-526C20	6,450,800–6,626,432	×2
RP11-222E10	6,560,463–6,759,394	×2
RP11-441C15	7,891,868–8,086,997	×2

[a]Positions of the BAC clones were based on the May 2004 (NCBI35/hg17) Human Assembly of the UCSC Genome Browser (http://genome.ucsc.edu/).

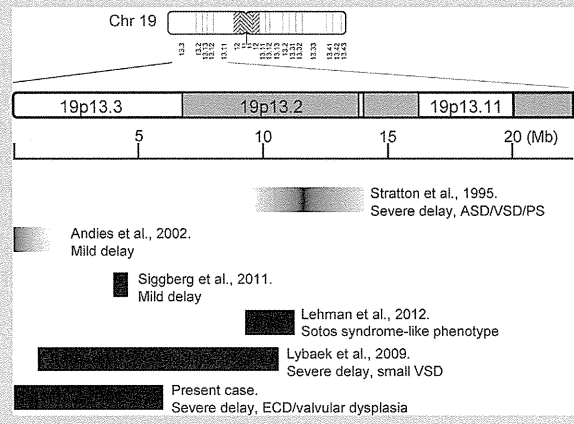

FIG. 3. Schematic representation of the microduplication on 19p13.3. The dark horizontal bars indicate the range of the duplication in reported patients. The duplicated regions in the patients reported by Stratton et al. [1995] and Andries et al. [2002] were ascertained from the respective reports.

TABLE II. Summary of Clinical Features in Five Individuals With Pure Microduplication at 19p13

	Stratton et al. [1995]	Andries et al. [2002]	Siggberg et al. [2011]	Lehman et al. [2012]	Lybaek et al. [2009]	Present patient
Age, sex	9 months, F	20 months, F	9 yrs, M	1–74 yrs, M/F	2 ½ yrs, F	3yrs, F
Karyotype	dup(19) (p13.2p13.13)	der(14)t (14;19) (q32.3;p13.3)	dup(19) (p13.3p13.3)	dup(19) (p13.2p13.2)	ins(19) (q13.3p13.2-p13.3)	der(10)t (10;19) (qter;p13.3)
Duplication	p13.2–p13.13	pter-p13.3	p13.3, 0.81 Mb	p13.2. 1.9 Mb	p13.3–p13.2, 8.9 Mb	p13.3, 6.10 Mb
(from pter)	?	?	(3.927–4.471 Mb)	(9.109–11.068 Mb)	(1.4–10.3 Mb)	(–6.106 Mb)
Pattern	Interstitial	Terminal	Interstitial	Interstitial	Interstitial	Terminal
Gestational age	Term	41 wks	Term	Term	35 wks	35 wks
Birth weight	2,730 g		2,730 g	4,550 g	1,790 g	1,216 g
Growth retardation	+	–	+	–	+	+ Severe
Development	Delay	Mild delay	Mild delay	Mild delay	Severe delay	Severe delay
Cardiovascular	PS, ASD, VSD	—	—	—	small VSD	ECD, PH, valvular dysplasia
Others	Strabismus, nail hypoplasia	Sparse hair, low-set ears, short nose	Amblyopia	Sotos syndrome-like	Severe eating problem, congenital hip dysplasia	Strabismus, renal aplasia (L), vertebral defects, nail hypoplasia, dislocation of hip joint

Thus, a duplication of >3 Mb of the terminal region of 19p13.3 might contribute to a more severe phenotype than do smaller duplications, and this phenotype might be characteristic of this chromosomal aberration.

Accurate assessment of the duplication size enabled us to evaluate the genes located within the duplicated region, which presumably contribute to the phenotypes. The duplicated region contains approximately 150 RefSeq genes and 130 OMIM genes, 18 of which have known disease associations. However, this case demonstrates that evaluation of the gene content of a chromosomal region is not sufficient to assess the pathogenicity of a gene duplication. Additional reports of individuals with this chromosomal aberration are required to demonstrate genotype–phenotype correlation in 19p duplication.

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ELSEVIER

Successful Endovascular Treatment of a Ruptured Superior Mesenteric Artery in a Patient with Ehlers–Danlos Syndrome

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The purpose of this study was to describe covered-stent treatment of a ruptured dissection of the superior mesenteric artery (SMA) in a patient with Ehlers–Danlos syndrome. The patient was a 13-year-old girl initially presenting with abdominal pain. Dissection and rupture of the SMA were diagnosed on detailed examination. Conservative treatment was performed initially because open surgery was considered high risk. However, the abdominal pain recurred, and we decided to perform endovascular therapy. A coronary artery covered stent was placed in the true lumen to close the entry site of the dissection. The false lumen was obliterated using a post-dilation technique, completing treatment of the rupture. The patient recovered uneventfully after surgery. Classic-type Ehlers–Danlos syndrome was diagnosed on the basis of physical findings and genetic analysis. The stent has remained adequately patent as of 2 years after surgery. This case report shows that dissection and rupture of the SMA can be treated successfully using a covered coronary artery stent in a patient with Ehlers–Danlos syndrome.

INTRODUCTION

Ehlers–Danlos syndrome (EDS) is comprised of a group of inherited connective tissue disorders, characterized by skin hyperextensibility, abnormal wound healing, and joint hypermobility. We describe our experience with a covered stent to repair a superior mesenteric artery (SMA) dissection that proceeded to rupture in a young girl with classic-type EDS. The patient recovered uneventfully, without the need for bowel resection.

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CASE REPORT

The patient was a 13-year-old girl who presented with abdominal pain. She had a history of scoliosis. The family history was irrelevant with regard to the current disorder. Abdominal pain suddenly developed after dinner, and a local physician was consulted. She was admitted because a computed tomography (CT) scan revealed retroperitoneal hemorrhage. On the following day, magnetic resonance imaging (MRI) was performed for further evaluation at another hospital. Dissection of the SMA was diagnosed, and the patient was transferred to our hospital.

On admission, her height was 140 cm and body weight 30 kg. The level of consciousness was alert. The patient had tenderness in the middle abdomen. An abdominal CT scan showed ruptured dissection of the SMA, leading to formation of a pseudoaneurysm. Because the portal vein was enhanced in the early phase, the pseudoaneurysm was considered to have perforated to the superior mesenteric vein (SMV) (Fig. 1).

In case of open surgery, SMA ligation with total resection of the small intestine may be required. In addition, the presence of a hereditary disease was suspected in this case, and emergent surgery was considered very risky. Because the patient's vital signs remained stable >24 hours after rupture, we therefore decided to apply

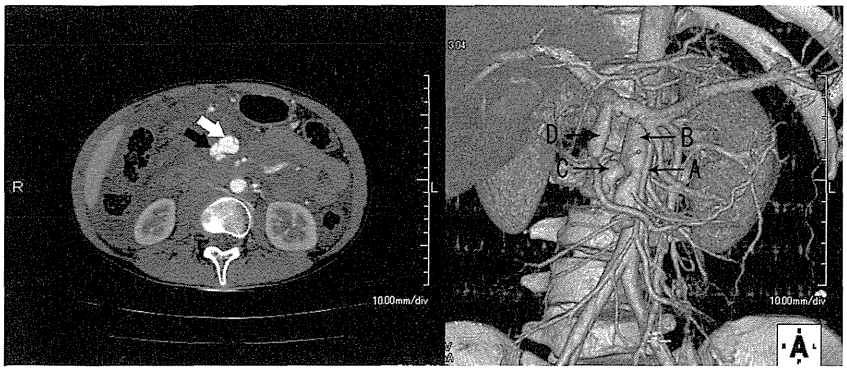

Fig. 1. CT scan (*left panel*) obtained at the time of presentation shows a retroperitoneal hematoma. An enlarged SMA with dissection and a patent false lumen (*white arrow*) and pseudoaneurysm (*black arrow*) were confirmed. A 3-dimensional CT scan (*right panel*) revealed the portal venous system in the arterial phase. Venous rupture of a pseudoaneurysm was diagnosed. **(A)** True lumen of the SMA. **(B)** False lumen of the SMA. **(C)** Pseudoaneurysm. **(D)** SMV. CT, computed tomography; SMA, superior mesenteric artery; SMV, superior mesenteric vein.

conservative therapy with close monitoring of the patient. Her abdominal pain improved by withdrawal of oral intake of food and liquids. A follow-up CT scan showed shrinkage of the retroperitoneal hematoma. Conservative management was abandoned because abdominal pain recurred on day 13, and CT scan revealed that the pseudoaneurysm had expanded. Entry closure of the dissection was therefore attempted by catheter intervention.

Arteriography of the SMA was performed percutaneously via the right femoral artery. The true lumen, false lumen, pseudoaneurysm, and blood flow to the SMV were confirmed. The entry site to the false lumen was also recognized (Fig. 2). Intravascular ultrasound (IVUS) was used during treatment to detect the entry site and the middle colic artery, and to measure true lumen diameter.

Because the true lumen of the SMA was only 3.2 × 5.2 mm, a device designed for coronary artery stent placement was used. Covered coronary stents are usually used to treat perforation of a coronary artery or saphenous vein graft at the time of percutaneous coronary intervention.[1] Under general anesthesia, a 6F sheath was inserted into the right brachial artery percutaneously. A 3.5 × 19-mm covered coronary stent graft (Jostent GraftMaster®; Abbott Vascular Japan, Tokyo, Japan) was delivered and dilated to occlude the entry. The stent consists of an ultrathin expandable, biocompatible polytetrafluoroethylene layer sandwiched between 2 coaxial 316 L stainless-steel stents. This covered stent can be used in vessels with diameters of 2.75–5.00 mm.[1] Because SMA angiography showed leakage of contrast medium into the false lumen, post-dilation was performed using of a 5 × 20-mm balloon dilation catheter (Sterling™; Boston Scientific, Natick, MA, USA). The leakage of contrast medium outside of the true lumen disappeared, indicating complete occlusion of the false lumen (Fig. 2).

There was no sign of intestinal necrosis, and the abdominal pain resolved. Oral intake was resumed on postoperative day 8. A CT scan on postoperative day 20 showed that the stent was patent without stenosis. Then, antiplatelet therapy with aspirin (100 mg/day) and ticlopidine hydrochloride (100 mg/day) was begun, with an expectation of long-term patency of the graft. However, the antiplatelet therapy had to be abandoned shortly thereafter due to persistent nasal bleeding. Ehlers–Danlos syndrome (EDS) was suspected 2 months after the intervention on the basis of physical findings, including hypermobility of the wrists and ankles, hallux valgus, a thin nasal crest, and multiple scars on the skin. Initially, we thought there was a possibility of vascular-type EDS. Full gene sequencing of all 51 exons and exon–intron boundaries of the *COL3A1* gene was performed, but no evidence of mutations in the gene was found. Classic-type EDS was finally diagnosed with further examination using next-generation sequencing technology, which revealed a 1-base mutation (c.1532G>T [G511V]) in exon 12 of the *COL5A1* gene. For this patient, it took a long time to diagnose EDS and establish a support system in the event of a recurrent emergency at home or in school, and therefore she was discharged 3 months after the intervention. Two years after discharge, the stent has remained patent (Fig. 3), and she is doing very well in high school.

DISCUSSION

Classic-type EDS is usually diagnosed clinically.[2] Forty-six percent of those with classic-type EDS have an identifiable mutation in *COL5A1* or *COL5A2*, the genes encoding type V collagen.[3] Classic-type EDS is inherited in an autosomal dominant manner. It is estimated that approximately 50% of affected individuals have inherited the disease-causing mutation from an affected parent and approximately 50% of affected individuals have a de novo disease-causing mutation.[2] In the

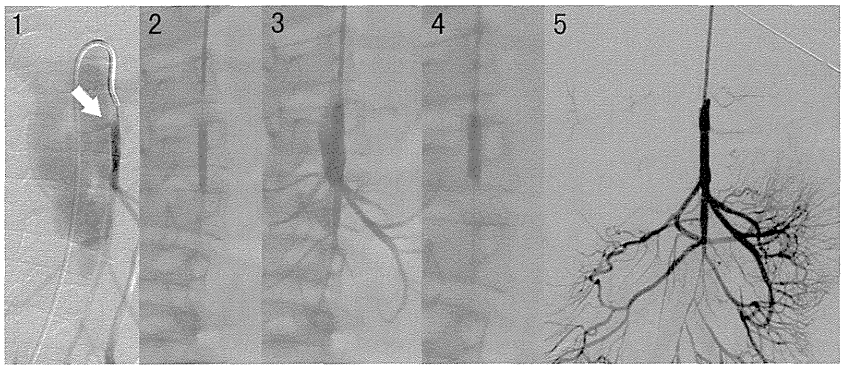

Fig. 2. (*1*) Angiography of the SMA confirmed an entry site in the false lumen (*white arrow*). (*2*) Dilation of a covered stent. (*3*) Angiogram obtained after stent placement, which shows that blood still flowed into the false lumen. (*4*) Post-dilation with a 5-mm balloon. (*5*) Final angiogram. There was no leakage of contrast medium outside of the true lumen, indicating that complete occlusion had been achieved.

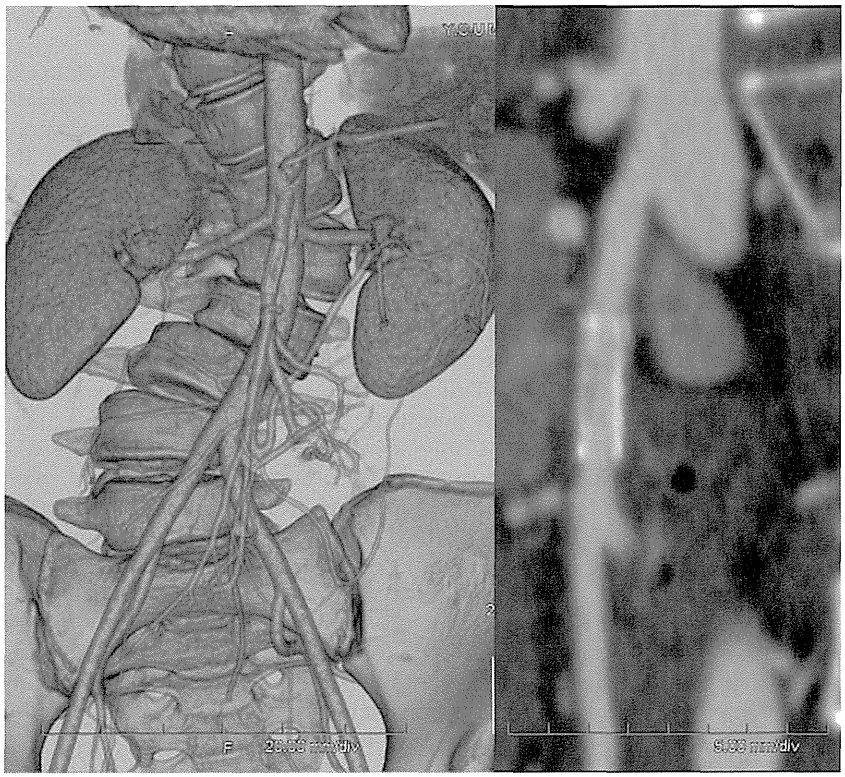

Fig. 3. CT scan obtained 2 years after surgery, showing that the stent remains well patent.

present case the gene mutation was proven; however, an additional examination for her parents was not performed due to their divorce. Although some cases have been reported,[4] it is rare that such a life-threatening arterial rupture would occur a patient with classic-type EDS.

Endovascular treatments for aneurysms and dissections have often been used to manage EDS type IV,[5–9] but few patients have undergone emergency catheter intervention.[10–13] Furthermore, catheter intervention has rarely been performed to treat SMA rupture in patients with EDS type IV.[14] To our knowledge, placement of a covered stent to manage SMA rupture has been reported in association with neurofibromatosis type 1[15] but not EDS. We chose endovascular therapy because serious hereditary disease was suspected in this patient. However, given the fact that her vascular

disease developed at 13 years of age, conventional vascular surgery was considered to carry a high risk of serious complications. Oderich et al. reported that vascular-type EDS was considered an increased risk for the development of late complications at the site of anastomosis of an artificial graft.[16] The patient described herein may not have had vascular-type EDS, but we believe that endovascular treatment should take priority over surgical treatment to avoid vascular complications. We emphasize that treatment was performed under general anesthesia, so that the patient could have been immediately converted to open surgery if the endovascular approach was unsuccessful.

There are some pitfalls in endovascular therapy for such patients with fragile blood vessels and connective tissue. Generally, in patients with small-caliber, fragile blood vessels, vascular puncture is difficult, and passage of a wire is problematic, often resulting in retrograde dissection. In such circumstances, ultrasound-guided puncture with an 11-MHz probe is useful. Hemostasis should be done very carefully. A false aneurysm may develop.

In our patient, an entry was present, but no reentry was found. This was attributed to the fact that a fistula had formed. We understand that extravasation can be completely controlled by major entry closure by stent grafting in patients with a ruptured type B aortic dissection. Even if there is a minor reentry site, the resulting shunt volume is usually minimal, and closure would occur. The only goal of treatment was therefore major entry closure. The placement site was simple to discern by using IVUS, and the stent was easily delivered with the use of a guiding catheter via the brachial artery. The stent expands to a maximum diameter of 5 mm with the expanded polytetrafluoroethylene membrane, resulting a shortening of the stent length, and therefore caution is required. In our patient, we successfully obliterated the false lumen by post-dilation with a 5-mm balloon catheter. There was no type II endoleak in the early phase. It remains unclear whether a type II endoleak developed in the false lumen in the delayed phase. Even if a leak that can be detected only on delayed-phase CT appears in the superacute phase after treatment, the branch would probably soon be obliterated because of the low blood flow.

After cardiovascular catheter interventions, subacute stent thrombosis has been reported to occur in 5.7% of patients, with a restenosis rate of 31.6%.[1] We hypothesized that the likelihood of stent thrombosis would be be much lower in the present case because the internal diameter of the stent was expanded up to 5 mm. Taking into account the risks of subacute thrombosis and rebleeding, we did not administer anticoagulation therapy in the early phase. Although gradual occlusion of the stent was predicted in this case, the stent has remained open. Close follow-up is required, which includes early detection of development of vascular lesions at other sites.

In conclusion, we have described the successful treatment of a ruptured dissection of the SMA with the use of a covered coronary artery stent in a patient with Ehlers–Danlos syndrome.

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CASE REPORT

Combination of Miller–Dieker syndrome and VACTERL association causes extremely severe clinical presentation

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Abstract We report a Japanese boy, who showed overlapping clinical features of Miller–Dieker syndrome (lissencephaly and facial dysmorphism) and vertebral defect, anal atresia, cardiac malformation and limb anomalies (VACTERL) association. The overall clinical presentation was much more severe than that normally associated with each disorder, and the infant died on day 100 of life despite aggressive therapy. Fluorescence in situ hybridization using a commercially available *LIS1* probe failed to detect a deletion, but chromosomal microarray analysis detected a 2.50-Mb microdeletion in 17p13.3 which involved partially the *LIS1* gene, and thus was compatible with Miller–Dieker syndrome. It may represent an example of a combination of two congenital disorders with blended phenotypes explaining unexpectedly severe phenotypes occurring with known chromosomal rearrangements. *Conclusion*: We report the first case of a combination of Miller–Dieker syndrome and VACTERL association with an unusually severe phenotype.

Keywords Miller–Dieker syndrome · 17p13.3 microdeletion · VACTERL association · Array comparative genomic hybridization

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Abbreviations

MDS	Miller–Dieker syndrome
FISH	Fluorescence in situ hybridization
NICU	Neonatal intensive care unit
CGH	Comparative genomic hybridization

Introduction

Miller–Dieker syndrome (MDS; MIM #247200) or 17p13.3 deletion syndrome is characterized by lissencephaly, intellectual disability, and facial dysmorphism [3]. Features of MDS include classically lissencephaly, microcephaly, wrinkled skin over the glabella and frontal suture, prominent occiput, narrow forehead, downward-slanting palpebral fissures, small nose and chin, cardiac malformations, growth retardation, and severe intellectual disability with seizures. The phenotype is attributed to haploinsufficiency of two genes present in the minimal critical region of MDS: *LIS1* (or *PAFAH1B1*; MIM #607432) and *YWHAE*; MIM #605066 [6]. MDS is commonly diagnosed by fluorescence in situ hybridization (FISH).

Vertebral defect, anal atresia, cardiac malformation and limb anomalies (VACTERL) association (MIM #276950) consists of a nonrandom combination of three or more of the following congenital defects: vertebral (V), anal (atresia, A), cardiac (C), tracheoesophageal (TE), radial and renal (R), and limb (L) abnormalities [8, 9]. A number of other defects have been reported to occur in addition to the originally described set, including genital anomalies, single umbilical artery, and caudal regression syndrome. The etiologies of VACTERL association remain largely unknown.

To our best of knowledge, having searched PubMed and Online Mendelian Inheritance in Man (OMIM) using such keywords as MDS and VACTERL association, we report here the first case of MDS accompanied by VACTERL

association. Genetic analysis revealed a microdeletion involving *LIS1* in 17p13.3 in a patient with an extremely severe clinical presentation including clinical and neuroradiological characteristics of both MDS and VACTERL association.

Case report

A Japanese boy was born at 37 weeks by vaginal delivery. He was the first child of the father. His mother, who had no history of exposure to any drugs, radiation, or infectious disease during her pregnancy, was 37 years old and had one healthy son with her former husband. The parents were Japanese, healthy, and nonconsanguineous, and there was no family history of congenital malformation or other relevant disease. The infant had a birth weight of 1,820 g (<3rd centile), a length of 38 cm (<7th centile), and a head circumference of 28 cm (<2nd centile), suggesting that he was small for gestational age. Fetal ultrasonography had shown intrauterine growth retardation. His Apgar scores at 1 and 5 min were 1 and 6, respectively. Meningocele, anal atresia, scoliosis, club feet, micropenis, cryptorchism, micrognathia, prominent occiput, small nose and chin, and low-set ears were noted at birth, and he was transferred to a secondary-referral neonatal intensive care unit (NICU) on the day of birth. Radiographs showed a butterfly vertebra (Fig. 1). Ultrasonography demonstrated double outlet right ventricle, patent ductus arteriosus, and ventricular septal defects. Head magnetic resonance imaging showed lissencephaly (Fig. 1). After repair of the meningocele and construction of a colostomy on day 1 of life, he was transferred to our tertiary-referral NICU on day 25 to undergo surgery for congenital heart disease.

Complete blood count and serum electrolytes, glucose, total protein, and transaminases were all normal. Serology was negative for toxoplasma, rubella, cytomegalovirus, and herpes simplex virus. On day 35, ductus arteriosus ligation and main pulmonary artery banding were performed. He developed pulmonary hypertension postoperatively, which was treated with inhaled nitric oxide. On day 94, he developed bilateral pneumothorax; hence, continuous aspiration was started on both sides. However, respiratory distress and multiorgan failure progressed despite aggressive therapy and the infant died on day 100 of life. Postmortem computed tomography and autopsy could not be performed because consent was not obtained from the parents. Conventional G-band chromosome analysis showed a normal karyotype of 46,XY. FISH was performed with commercially available probes (Miller Dieker (*LIS1*) Probe Combination Aquarius

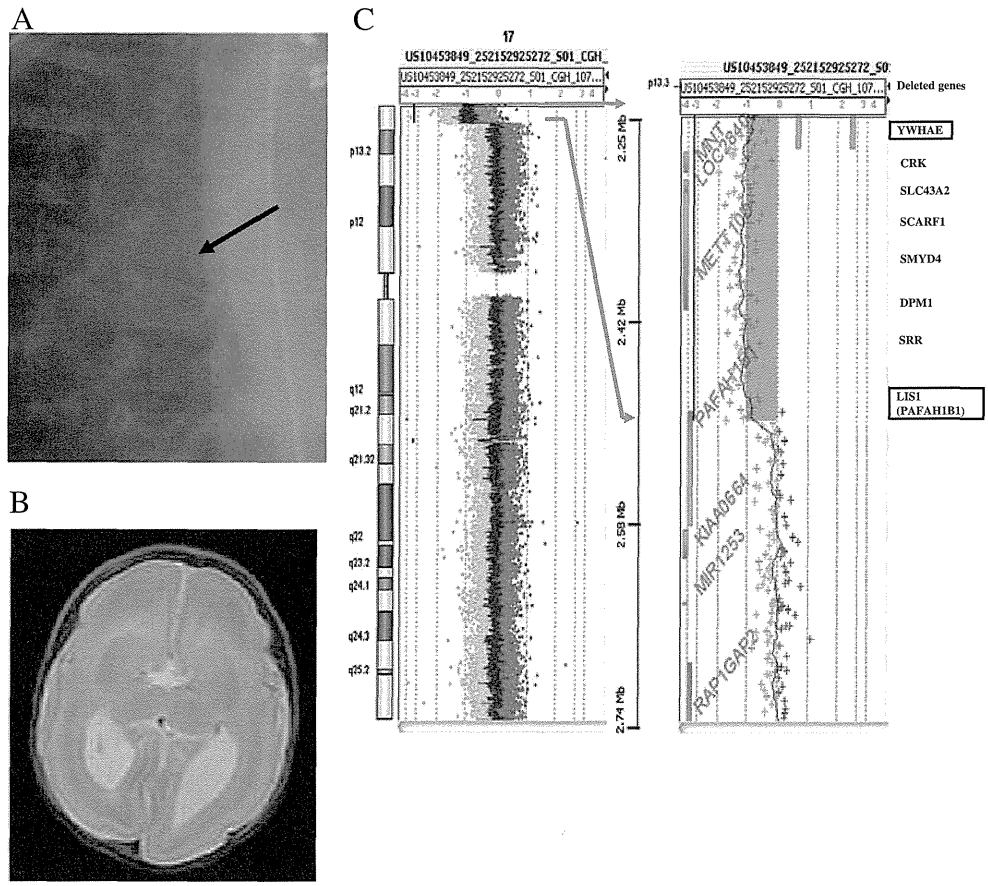

Fig. 1 **a** Butterfly vertebra (*arrow*), **b** lissencephaly as visualized by MRI, and **c** array comparative genomic hybridization showed a deletion of 2.5 Mb in 17p13.3 which involved the *YWHAE* gene and part of the *LIS1* gene

(SRL, Tokyo, Japan)), using standard protocols that revealed no deletion in *LIS1*. Table 1 shows a comparison of typical features of VACTERL association and Miller–Dieker syndrome and their clinical presentation in the current patient.

Methods

After obtaining approval of the ethics committees of Nagoya City University and Kanagawa Children's Medical Center and written informed consent from the parents, genetic analysis of the infant was performed. Genomic DNA was extracted from the patient's peripheral blood using the QIAquik DNA extraction kit (QIAGEN, Tokyo, Japan). Array comparative genomic hybridization (CGH) was performed using Agilent Sure Print G3 Human CGH Microarray 1 M (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's protocol. As control DNA, commercially available male genomic DNA (Promega, Madison, WI, USA) was used. Microarrays were analyzed using Agilent Features Extraction Software. Graphical overviews and analysis of the data were obtained with the Agilent CGH Analytic software.

Results

Array CGH analysis showed a deletion of 2.50 Mb in 17p13.3 as arr 17p13.3(47,546-2,504,386)x1. The deletion involved the *YWHAE* gene and part of the *LIS1* gene (Fig. 1c). Genetic analysis of the infant's parents and half-brother could not be studied because consent was not obtained.

Discussion

We report the first case of a combination of MDS and VACTERL association. The patient demonstrated an extremely severe clinical presentation including features of both disorders (Table 1). Genetic testing was performed to help diagnose the associated malformations. The karyotype was 46,XY without any obvious deletions. In addition, FISH revealed no deletion in 17p13.3, the region responsible for MDS. However, array CGH identified a 2.5-Mb deletion in 17p13.3 and a microdeletion in a region of *LIS1*. Initial FISH study using a commercially available *LIS1* probe failed to detect the microdeletion because it involved only exon 1 of *LIS1*. These findings were consistent with MDS and 17p13.3 microdeletion, including the clinical features of lissencephaly, facial dysmorphism, cardiac malformations, and fetal growth retardation [6]. Similarly, Izumi et al. reported a patient with classic MDS who received a false-negative FISH result when analysis was performed using a widely used commercial FISH probe [5]. However, the present patient had very severe disease with complications not typically seen in MDS, and was not able to survive despite aggressive treatment. The other findings, including butterfly vertebra, anal atresia, scoliosis, limb anomalies, micropenis, and bilateral cryptorchism, were difficult to explain by MDS alone.

On the other hand, the VACTERL association refers to an acronym for a combination of vertebral defects, anal atresia, cardiac, tracheoesophageal fistula with esophageal atresia, radial and renal dysplasia, and limb abnormalities [8, 9]. The diagnostic criteria require at least three of these findings [9]. Our patient fulfilled the diagnostic criteria for a VACTERL association, with butterfly vertebra, anal atresia, double outlet right ventricle, and limb anomalies (Table 1). A search of

Table 1 A comparison of features of VACTERL association and Miller Dieker syndrome and their clinical presentation in the current patient

	VACTERL association	Miller–Dieker syndrome	Current patient
Vertebral defects	+	−	+
Anal atresia	+	−	+
Cardiac malformation	+	+	+
Tracheoesophageal fistula	+	−	−
Esophageal atresia	+	−	−
Radial dysplasia	+	−	−
Renal dysplasia	+	−	−
Limb anomalies	+	−	+
Genital anomalies	+	−	+
Lissencephaly	−	+	+
Facial dysmorphism	−	+	+
Feeding difficulties	−	+	+
Development disorder	−	+	+
Fetal growth retardation	−	+	+

PubMed and OMIM using such keywords as MDS and VACTERL association suggested that MDS with VACTERL association has not previously been reported.

The etiology of VACTERL association is currently largely unknown. The disorder might be heterogeneous and could have several genetic or non-genetic causes [9]. It has been linked to abnormal mesodermal development and differentiation, and it is thought to involve the *SHH* (MIM #600725) [2] and *FOXF1* (MIM #601089) genes [1]. Moreover, administration of adriamycin to chicken eggs can trigger a phenotype similar to that seen in the VACTERL association in the resulting chicks [7]. Therefore, environmental factors may play an important role in the pathogenesis of VACTERL association. Such environmental factors are likely to disturb normal gene expression, and could perturb precise gene expression patterns during embryogenesis, resulting in VACTERL phenotype. In this patient, a combination of the genetic factor of 17p13.3 microdeletion and an unknown factor causing VACTERL association may have given rise to the unusually severe clinical presentation that included pulmonary hypertension after cardiac surgery.

Recently, a two-hit model for severe phenotypes due to an additional second gene abnormality ("second hit") has been proposed for microdeletion syndromes [10]. Girirajan et al. reported the development of neuropsychiatric symptoms with an isolated 16p12.1 microdeletion, in which the clinical presentation was more severe due to copy number variations as an additional second hit [4]. The second hit may not be a genetic factor, but could be an environmental or epigenetic factor. Our patient had a very severe clinical course with an MDS phenotype despite a small microdeletion that could not be detected by FISH. Array CGH analysis showed no additional pathogenic copy number variations other than the 17p13.3 microdeletion as a second hit in this patient. Therefore, the original "second hit model" cannot be applied to our patient. Nevertheless, it is possible that an unknown etiological factor causing VACTERL association modified the 17p13.3 microdeletion phenotype, resulting in a much more severe phenotype. We need to be very careful in interpreting the phenotypic consequence of any given genetic mutations because a modifying etiological factor may not be identified by routine examination.

This study has several limitations. The first is that only one patient is reported, so further accumulation of similar cases is necessary. Moreover, genetic testing could not be performed in the parents or elder half-brother. Thus, we do not know whether there were parental chromosome abnormalities such as translocations. However, based on clinical severity in this patient and the fact that the mother later gave birth to a healthy child, this is most likely a sporadic case. To the best of our knowledge, this is the first reported case of a combination of Miller–Dieker syndrome and VACTERL association. It may represent an example of a combination of two congenital disorders with blended phenotypes explaining unexpectedly severe phenotypes occurring with known chromosomal rearrangements.

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Conflict of interest The authors have no conflicts of interest.

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