雑　誌（英文）

CLINICAL REPORT

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Neurodevelopmental Features in 2q23.1 Microdeletion Syndrome: Report of a New Patient With Intractable Seizures and Review of Literature

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2q23.1 microdeletion syndrome is a recently characterized chromosomal aberration disorder uncovered through array comparative genomic hybridization (array CGH). Although the cardinal feature is intellectual disability (ID), neurodevelopmental features of the syndrome have not been systematically reviewed. We present a 5-year-old boy with severe psychomotor developmental delay/ID, progressive microcephaly with brain atrophy, growth retardation, and several external anomalies. He manifested intractable epilepsy, effectively treated with combined antiepileptic drug therapy including topiramate. Array CGH demonstrated a de novo interstitial deletion of approximately 1 Mb at 2q23.1–q23.2, involving four genes including *MBD5*. Nineteen patients have been reported to have the syndrome, including present patient. All patients whose data were available had ID, 17 patients (89%) had seizures, and microcephaly was evident in 9 of 18 patients (50%). Deletion sizes ranged from 200 kb to 5.5 Mb, comprising 1–15 genes. *MBD5*, the only gene deleted in all patients, is considered to be responsible for ID and epilepsy. Furthermore, the deletion junction was sequenced for the first time in a patient with the syndrome; and homology of three nucleotides, identified at the distal and proximal breakpoints, suggested that the deletion might have been mediated by recently-delineated genomic rearrangement mechanism Fork Stalling and Template Switching (FoSTeS)/microhomology-mediated break-induced replication (MMBIR).

Key words: 2q23.1 microdeletion syndrome; array CGH; neurological features; epilepsy; *MBD5*; FoSTeS/MMBIR

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INTRODUCTION

The 2q23.1 microdeletion syndrome is a recently characterized chromosomal aberration disorder uncovered by array comparative genomic hybridization (array CGH). To date, only 18 patients have been reported to have the syndrome (Table I) [Vissers et al., 2003; Koolen et al., 2004; de Vries et al., 2005; De Gregori et al., 2007; Wagenstaller et al., 2007; Jaillard et al., 2009; Williams et al., 2010; van Bon et al., 2010; Chung et al., 2011]. The cardinal feature is intellectual disability (ID) with pronounced speech delay. Additional features include coarse face, short stature, microcephaly, seizures, and behavioral abnormalities such as stereotypic

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TABLE I. Neurological and Molecular Findings of Our Patient and 18 Previously Reported Patients With Microdeletion Encompassing the 2q23.1 Region

			Deleted region						Seizures					
Patient [reference]	Sex	Array	Start	End	Size (Mb)	Deleted genes	OFC (centile)	MR	Severity	Type	Onset	AEDs	EEG	MRI
Our patient	M	Agilent Oligo 1M	148.8	149.8	0.992	*MBD5, EPC2, KIF5C, LYPD6B*	<3rd (−3.6 SD)	Severe	Severe	CPS	10M	CBZ, ZNS, CLB, TPM	$F^{\#1}$	Myelination delay, brain atrophy in $F^{\#1}$ and $T^{\#2}$
1 [Wagenstaller et al., 2007, Patient 27737]	M	Affimetrix SNP 100K	149.0	149.2	0.2	*MBD5*	ND	Severe	Drug resistant	ND	1Y4M	ND	ND	ND
2 [Jaillard et al., 2009, Subject 1]	M	Agilent Oligo 44K	148.8–149.1	149.3–149.4	0.3	*MBD5, EPC2*	<3rd (−3 SD)	Severe	ND	ND	3M	ND	Non-specific	Small cerebellar vermis
3 [van Bon et al., 2010, Patient 8a]	M	Agilent Oligo 244K	148.5	148.9	0.4	*ORC4L, MBD5*	25th	Moderate	—	—	—	—	ND	Normal
4 [van Bon et al., 2010, Patient 8b]	F	Agilent Oligo 244K	148.5	148.9	0.4	*ORC4L, MBD5*	25th	Moderate	ND	ND	10Y	ND	ND	Ventricular asymmetry
5 [van Bon et al., 2010, Patient 7]	M	Agilent Oligo 44K	148.7	149.2	0.5	*MBD5, EPC2*	<3rd	ND	Severe epileptic encephalopathy	GTCS, GTS, AS, Ab	3Y	Multiple	Multiregion	Normal
6 [Williams et al., 2010, Case 1]	F	Agilent Oligo 244K	148.4	149.4	~0.93	*ORC4L, MBD5, EPC2, KIF5C*	<3rd	ND	Well controlled	CPS	8Y	OXC	Mild diffuse encephalopathy changes	Normal
7 [van Bon et al., 2010, Patient 10]	M	Agilent Oligo 244K	148.1	149.2	1.1	*ACVR2A, ORC4L, MBD5, EPC2*	<3rd	Severe	ND	PS, Secondary GS	Newborn	ND	$F^{\#1}$ and $C^{\#3}$	Focal cortical abnormalities in right $T^{\#2}$
8 [van Bon et al., 2010, Patient 9]	F	Affymetrix SNP 250K	148.8	150	1.2	*MBD5, EPC2, KIF5C, LYPD6B, LYPD6*	10th	Severe	Drug resistant	ND	10M	ND	Right $T^{\#2}$ and $O^{\#5}$	Wide frontal ventricles and myelination delay
9 [Koolen et al., 2004]	F	BAC 3.6K	145.4–146.7	148.7–151.1	2.0	*PABPCP2, ACVR2A, ORC4L, MBD5, EPC2, KIF5C, LYPD6B*	<3rd (−2 SD)	Severe	ND	GS	12Y	ND	ND	Cortical atrophy
10 [van Bon et al., 2010, Patient 5]	F	Agilent Oligo 244K	148.4	151.1	2.7	*ACVR2A, ORC4L, MBD5, EPC2, KIF5C, LYPD6B, LYPD6, MMADHC, RND3*	50th	Severe	Drug resistant, and died after several seizures	ND	9M	ND	ND	ND
11 [van Bon et al., 2010, Patient 2]	F	Affymetrix SNP 250K	148.7	151.5	2.8	*MBD5, EPC2, KIF5C, LYPD6B, LYPD6, MMADHC, RND3*	10th	ND	ND	ND	3Y10M	ND	ND	Normal

12 [van Bon et al., 2010, Patient 3]	F	Agilent Oligo 244K	148.1	151	2.9	*ACVR2A, ORC4L, MBD5, EPC2, KIF5C, LYPD6B, LYPD6, MMADHC*	50th	Moderate	—	—	—	—	ND	Normal
13 [van Bon et al., 2010, Patient 4]	M	HumanCNV370 CNV-SNP 370K	147.2	150.1	2.9	*ACVR2A, ORC4L, MBD5, EPC2, KIF5C, LYPD6B, LYPD6*	16th	Severe	ND	AS	1Y5M	ND	ND	Thinnng of PCC$^{\#4}$
14 [Jaillard et al., 2009, Subject 2]	M	IntegraChips BAC 3K	145.3–146.9	149.3–150.7	2.4–5.4	*PABPCP2, ACVR2A, ORC4L, MBD5, EPC2, KIF5C, LYPD6B, LYPD6, MMADHC*	Median	Severe	ND	ND	3Y	VPA	Normal	Hypoplasia of F$^{\#1}$
15 [Williams et al., 2010, Case 2]	F	Agilent Oligo 244K	146.8	150.3	3.51	*PABPCP2, ACVR2A, ORC4L, MBD5, EPC2, KIF5C, LYPD6B, LYPD6, MMADHC*	15th	ND	Well controlled	GTCS	8M	VPA	Light F$^{\#1}$	Normal
16 [Chung et al., 2011]	F	Agilent Oligo 105K/244K	148.9	152.9	3.986	*MBD5, EPC2, KIF5C, LYPD6B, LYPD6, MMADHC, RND3, RBM43, NMI, TNFAIP6, RIF1, NEB, ARL5A, CACNB4, STAM2*	<3rd	Moderate, regression at age 6 years	Well controlled	ND	3Y	ND	ND	Normal
17 [van Bon et al., 2010, Patient 6]	M	Cytochip v 3.01 BAC >5K	146.7	151.8	5.2	*PABPCP2, ACVR2A, ORC4L, MBD5, EPC2, KIF5C, LYPD6B, LYPD6, MMADHC, RND3*	<3rd	ND	ND	Ab, Fs	2Y	ND	Normal	Normal
18 [van Bon et al., 2010, Patient 1]	F	Affymetrix SNP 500K	146.6	152.2	5.5	*PABPCP2, ACVR2A, ORC4L, MBD5, EPC2, KIF5C, LYPD6B, LYPD6, MMADHC, RND3, RBM43, NMI, TNFAIP6, RIF1, NEB*	2nd	ND	ND	ND	ND	ND	MD	White matter abnormalities

MR, mental retardation; AEDs, antiepileptic drugs; ND, not described; CPS, complex partial seizure; AS, atonic seizure; Ab, absence seizure; Fs, febrile seizure; GTCS, generalized tonic-clonic seizure; GTS, generalized tonic seizure; GS, generalized seizure; Secondary GS, secondary generalized seizure; PS, partial seizure; CBZ, carbamazepine; ZNS, zonisamide; CLB, clobazam; TPM, topiramate; VPA, valproate; OXC, oxcarbazepine.
F$^{\#1}$, frontal region; T$^{\#2}$, temporal region; C$^{\#3}$, central region; PCC$^{\#4}$, posterior corpus callosum; O$^{\#5}$, occipital region.

repetitive behavior, disturbed sleep pattern, and broad-based gait [van Bon et al., 2010].

There have been no reports reviewing neurodevelopmenal features in 2q23.1 microdeletion syndrome. We here present the detailed clinical features and course of a boy with the syndrome who had severe psychomotor developmental delay and ID, progressive microcephaly, and intractable epilepsy that was improved by multi-drug therapy including topiramate (TPM). High-resolution array CGH demonstrated a 992-kb deletion at 2q23.1–q23.2 involving four genes including *MBD5*, and the breakpoint-junction sequencing revealed microhomology of three nucleotides at the distal and proximal breakpoints, suggesting that the deletion might have been mediated by recently delineated genomic rearrangement mechanism Fork Stalling and Template Switching (FoSTeS)/microhomology-mediated break-induced replication (MMBIR).

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The patient is the third child, with two healthy brothers, of a healthy non-consanguineous 33-year-old mother and 34-year-old father. He was born at 38 weeks of gestation by spontaneous vaginal delivery. His birth weight was 2,960 g (−0.1 SD), length 48.2 cm (−0.2 SD), and OFC 31.5 cm (−1.0 SD). He has suffered from bronchial asthma since infancy. At the age of 10 months, he was referred to our hospital for afebrile clonic seizures involving alternating sides with impaired consciousness. The patient's seizures tended to occur episodically and in clusters. Although he had one episode of status epilepticus for 60 min, most of his seizures were not prolonged. On presentation, his weight, height, and OFC were 9.1 kg (± 0 SD), 71 cm (−0.7 SD), and 42.5 cm (−2.0 SD, Fig. 1a), respectively. He showed psychomotor delay, with a developmental quotient (DQ) of 57 on the Kinder Infant Development Scale (KIDS) [Cheng et al., 2010]. His craniofacial features included brachycephaly, strabismus, a short nose with anteverted nostrils, a short philtrum, macroglossia, a high palate, a bifid uvula, and a submucous cleft palate. Additionally, he had short and curved 5th fingers, a single transverse crease on the right palm, and bilateral undescended testes. Blood levels of lactate and pyruvate and serum levels of thyroid hormones were within normal ranges. Amino acid and organic acid disorders were excluded. A cardiac ultrasonography showed mild supravalvular pulmonary artery stenosis. An interictal EEG showed sporadic spikes in the frontal region during sleep with normal background activity (Fig. 1b). A brain MRI showed no obvious abnormalities and a normal myelination pattern.

The patient's clinical course of epilepsy is shown in Fig. 1c. Carbamazepine (CBZ) was started at the age of 10 months. Seizure frequency and duration decreased, but he began exhibiting stereotypical characteristics of frontal lobe epilepsy (FLE): motion and speech arrested with mild rigidity of the upper limbs and incomplete loss of consciousness for 40–60 sec. Zonisamide (ZNS) resulted in a slight reduction of seizure frequency and clobazam (CLB) controlled his condition for several months, although the seizures restarted with cluster attacks. At the age of 4 years, TPM altered from ZNS reduced his seizure frequency and intensity, disappeared cluster attacks, and shortened (<30 sec) the durations. An interictal EEG showed many paroxysmal discharges with slow wave in the frontal region (Fig. 1d). A brain MRI at the age of 5 years showed delayed myelination and mild brain atrophy (Fig. 1e).

Microcephaly was evident after age 1 (Fig. 1a). The patient could roll over and sit without support at the age of 9 months, walk independently at 21 months, and jump at 4 years. When last examined at the age of 5 years, he weighed 15.2 kg (−1.0 SD) and had a height of 98.3 cm (−2.0 SD), and OFC of 45.3 cm (−3.4 SD). He could produce several words but no sentences. Although he had no regressive psychomotor changes, his DQ as evaluated by KIDS had dropped to 24 from 46 at age 2.

CYTOGENETIC AND MOLECULAR ANALYSIS

G-banded chromosomal analysis (550 bands level) using the patient's peripheral blood leukocytes showed a normal karyotype (46,XY). Array CGH analysis with Agilent 1M array (Agilent Technologies, Inc., Santa Clara, CA) demonstrated a 992-kb heterozygous deletion at 2q23.1–q23.2 (USCS hg18, Mar. 2006, chromosome 2: 148,830,937–149,823,345 bp) (Fig. 2a). The deletion was confirmed with FISH using probes originated from four BACs (RP11-295N18 at 2q22.3, RP11-375H16 at 2q23.1, RP11-1005D13 at 2q23.2, and RP11-714O10 at 2q23.3): RP11-375H16 and RP11-1005D13 were deleted, whereas RP11-295N18 and RP11-714O10 were present (Fig. 2b). Subsequently, the deletion junction was amplified by a long PCR (Fig. 2c; sequences of the primer set are available on request) and its product was directly sequenced (Fig. 2d). Three nucleotides (CTG) were shared by sequences at the proximal breakpoint and the distal breakpoint in normal chromosome 2 (Fig. 2d). FISH analysis using the four BAC probes (data not shown) and the junction PCR on parental samples (Fig. 2c) showed that the deletion had occurred de novo. Therefore, the karyotype was concluded as 46,XY.arr 2q23.1q23.2-(148,830,937–149,823,345) × 1 dn, and he was diagnosed with 2q23.1 microdeletion syndrome involving four genes: *MBD5*, enhancer of polycomb, drosophila, homolog of 2 (*EPC2*), kinesin family member 5C (*KIF5C*), and LY6/PLAUR domain containing 6B (*LYPD6B*).

DISCUSSION

The patient we have described had severe psychomotor developmental delay and ID with progressive microcephaly and intractable seizures improved by multi-drug therapy including TPM. Roughly 1 Mb region at 2q23.1–q23.2, which involved four genes including *MBD5*, was found to be deleted and both the proximal and the distal breakpoints were demonstrated to share microhomology of three nucleotides.

The neurological and molecular cytogenetic findings of 19 patients with the syndrome, including the present patient, are shown in Table I. Microcephaly (OFC < 3rd centile) was evident in 9 of 18 patients (50%), whereas younger patients with OFC > 3rd centile might exhibit microcephaly thereafter as demonstrated in the present patient. Moderate to severe ID was noted in all patients whose data were available. Seventeen patients (89%) had seizures: generalized seizures in five patients, partial seizures in three, and seizures of unspecified nature in nine. Median age of seizure onset

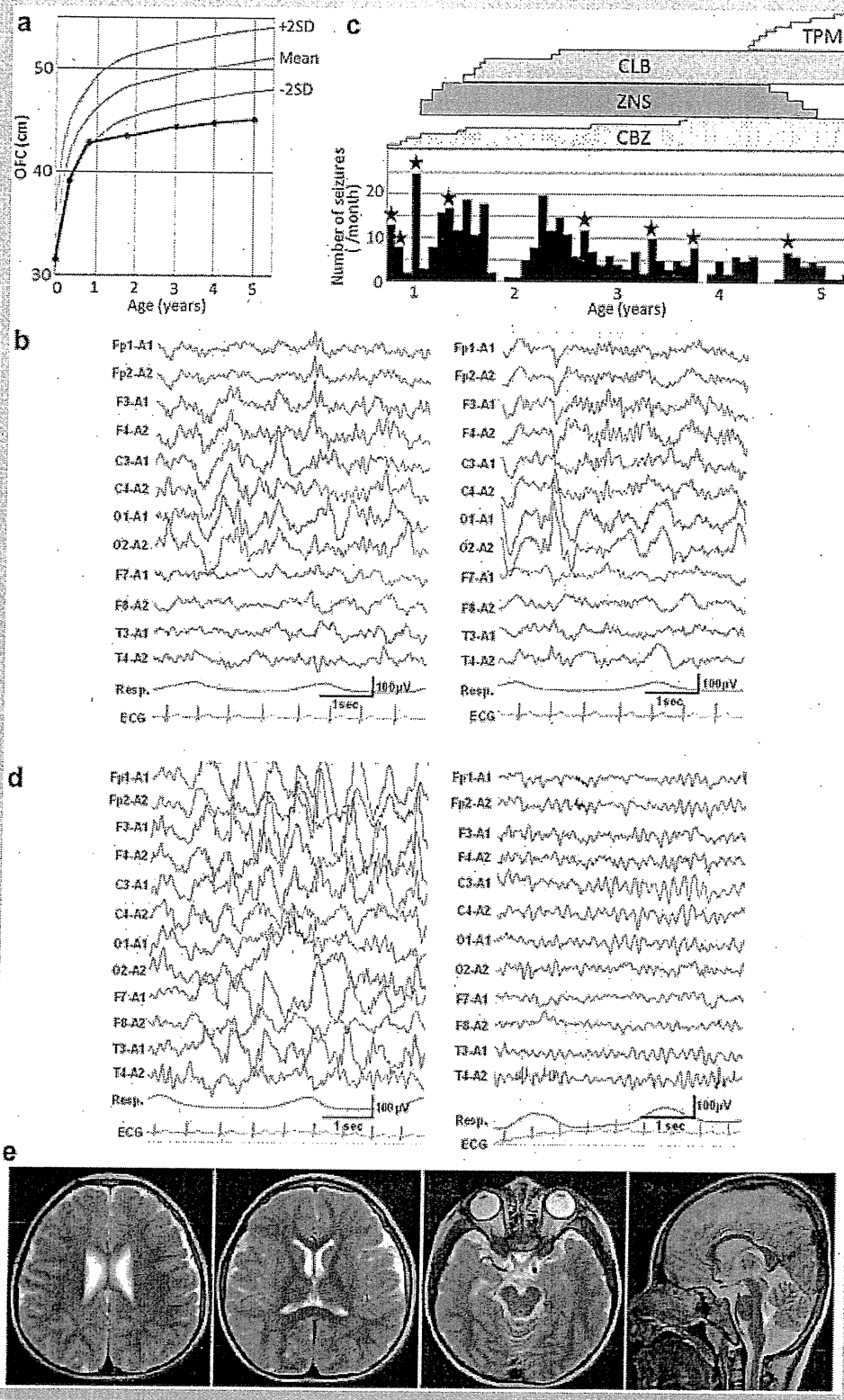

FIG. 1. Clinical findings. a: OFC growth curve. b: An interictal EEG during sleep at age 10 months. Sporadic spikes over the frontal region during sleep records are observed (left). Background activity is consistent with his age (right). c: Therapeutic course of seizures. CBZ, carbamazepine; ZNS, zonisamide; CLB, clobazam; TPM, topiramate. ★ indicates seizures with cluster attacks. d: An interictal EEG at age 4 years. Many paroxysmal discharges including not only sporadic spikes but also slow wave bursts in the bilateral frontal region during sleep are observed (left). A lower frequency theta rhythm in the front-central region during wakefulness is recorded (right). e: Brain MRI at age 5 years. Delayed myelination and mild brain atrophy mainly in the front-temporal lobe are noted.

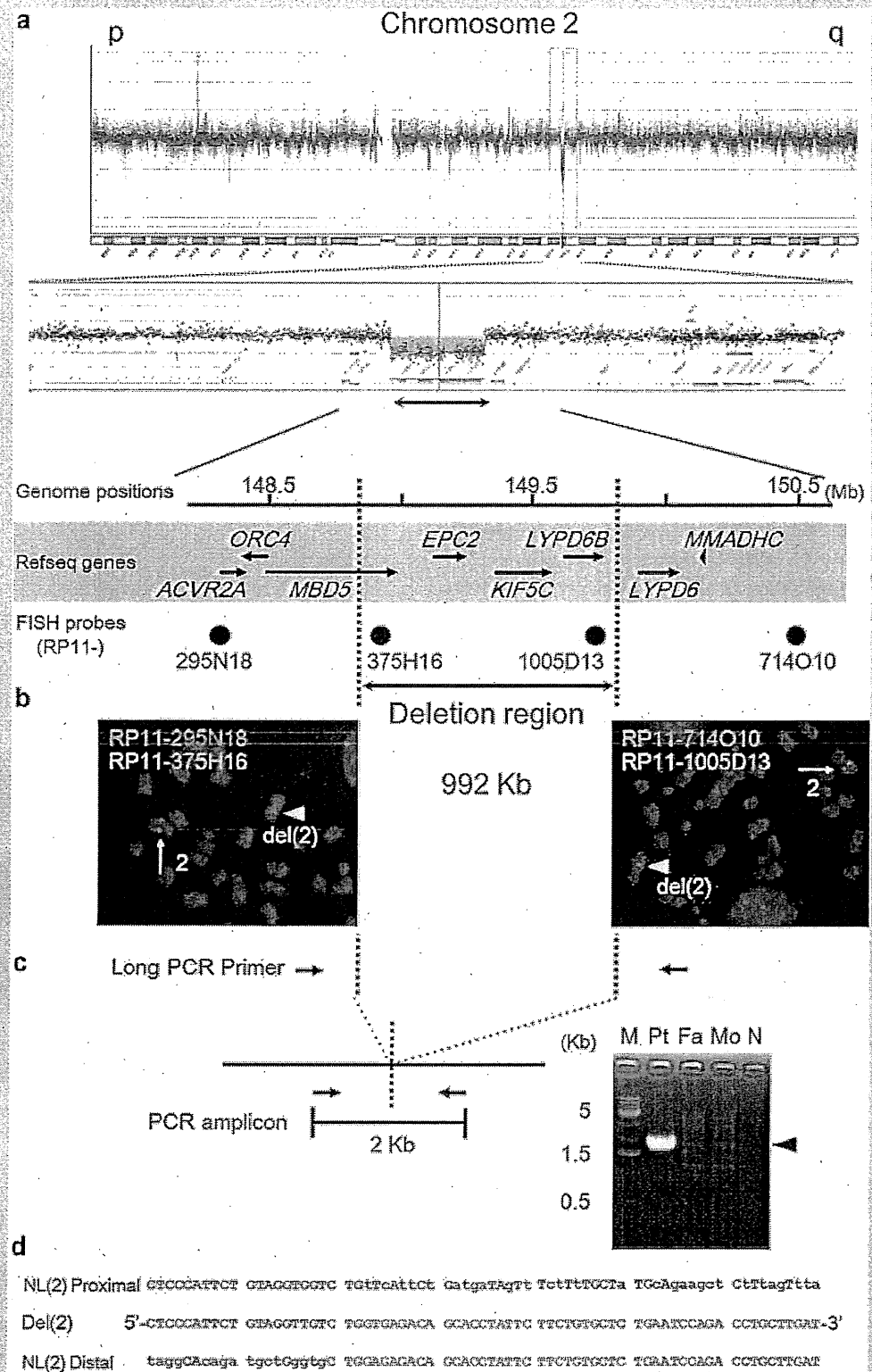

FIG. 2. Cytogenetic and molecular findings. a: High-resolution oligo array [Agilent 1M array] showing the 992 Kb deletion at 2q23.1–q23.2 that contains three Refseq genes [*EPC2*, *KIF5C*, *LYPD6B*] and a disrupted *MBD5*. b: FISH analyses using probes for four BACs [RP11-295N18, RP11-375H16, RP11-1005D13, RP11-714O10]. A normal chromosome 2 [2] is indicated by white arrows and a deleted chromosome 2 [del(2)] is indicated by white arrowheads. Signals for the RP11-375H16 and RP11-1005D13 probes are not detected on deleted chromosome 2. c: Deletion junction PCR showing that an aberrant 2 Kb-PCR amplicon is detected only in the patient [black arrowhead]. M, size markers; Pt, the patient's sample; Fa, the father's sample; Mo, the mother's sample; N, negative control. d: Deletion junction sequence. The normal upper and lower sequences are seen around the proximal [2q23.1] and the distal [2q23.2] deletion breakpoints, respectively. Homologous sequences are indicated by capital letters. The middle sequence is the deletion junction of the patient. NL(2), normal chromosome 2; Del(2), deleted chromosome 2.

was 1 years and 8.5 months (range, neonate to 12 years old). Although the seizures of three patients were well controlled, those of five patients were intractable. In particular, patient 10 began suffering from seizures at the age of 9 months and died at 26 years because of seizures. Patient 5 developed severe epileptic encephalopathy. EEG abnormalities were found in seven of nine patients (78%). The present patient was considered to have FLE with complex partial seizures. His seizures were multi-drug resistant and EEG findings progressively worsened. The effect of CLB was transient, and TPM appeared to be most effective at present to control seizures. MRI abnormalities were detected in 9 of 17 patients (53%) and included cortical atrophy, small vermis, cortical or subcortical lesion, ventricular asymmetry, thinning of the posterior corpus callosum, and delayed myelination. Interestingly, the present patient had no obvious abnormalities during infancy, but later showed brain atrophy and delayed myelination in the front-temporal region at the age of 5 years that were consistent with EEG findings. Considering such a progressive course, careful longitudinal observation of neurodevelopmental features would be necessary for this syndrome. The chromosomal deletion size in the syndrome ranged from 200 kb to 5.5 Mb, but did not seem to correlate with severity of ID, seizures, or MRI abnormalities. Since deleted regions comprised only a part of *MBD5* to 15 genes including *MBD5*, *MBD5* is considered to be responsible for moderate to severe ID and well-controlled to intractable epilepsy.

The deletions in the patients with this syndrome have not been considered to be mediated by NAHR, because the reported deletions did not have common breakpoints [van Bon et al., 2010]. In this study, we sequenced the deletion junction for the first time. The proximal breakpoint was located within a LINE element (LIP3) included in the genomic region of *MBD5*, whereas the distal breakpoint was not located within a LINE element, according to UCSC genome browser. BLAST search found no significant sequence similarity between LIP3 and a sequence around the distal breakpoint in normal chromosome 2. For NAHR to take place, there must be segments of a minimal length sharing extremely high similarity or identity between the low copy repeats, with 10–400 kb in length and >96% sequence identity [Gu et al., 2008]. We, therefore, have concluded the deletion in the present patient not to be mediated by NAHR. Furthermore, we have identified a consensus sequence CTG both at the proximal and the distal breakpoints. This sequence microhomology might have resulted in the deletion in the present patient, via the recently delineated genomic rearrangement mechanism FoSTeS/MMBIR. The mechanism has been reported to mediate genomic rearrangements in Pelizaeus-Merzbacher disease [Lee et al., 2007], Potoki-Lupski microduplication syndrome [Zhang et al., 2009], Smith-Magenis microdeletion syndrome [Zhang et al., 2009], and Charcot-Marie-Tooth disease type 1A duplication/hereditary neuropathies with liability to pressure palsies [Zhang et al., 2009], as well as in 87% of patients with rare pathologic copy number variations [Vissers et al., 2009]. Thorough investigation of breakpoint sequences in the other patients would uncover the etiology of deletions of 2q23.1 microdeletion syndrome.

In conclusion, the present study described detailed neurodevelopmental features including the therapeutic course for intractable epilepsy in a patient with 2q23.1 microdeletion syndrome. Review of neurological and molecular features in previously reported patients demonstrated that *MBD5* would be responsible for ID and epilepsy. Microhomology of three nucleotides, identified at the distal and proximal breakpoints, suggested that the deletion might have been mediated by recently delineated genomic rearrangement mechanism FoSTeS/MMBIR.

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CLINICAL REPORT

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Myelodysplastic Syndrome in a Child With 15q24 Deletion Syndrome

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15q24 deletion syndrome is a recently-described chromosomal disorder, characterized by developmental delay, growth deficiency, distinct facial features, digital abnormalities, loose connective tissue, and genital malformations in males. To date, 19 patients have been reported. We report on a 13-year-old boy with this syndrome manifesting childhood myelodysplastic syndrome (MDS). He had characteristic facial features, hypospadias, and mild developmental delay. He showed neutropenia and thrombocytopenia for several years. At age 13 years, bone marrow examination was performed, which showed a sign suggestive of childhood MDS: mild dysplasia in the myeloid, erythroid, and megakaryocytic cell lineages. Array comparative genomic hybridization (array CGH) revealed a de novo 3.4 Mb 15q24.1q24.3 deletion. Although MDS has not been described in patients with the syndrome, a boy was reported to have acute lymphoblastic leukemia (ALL). The development of MDS and hematological malignancy in the syndrome might be caused by the haploinsufficiency of deleted 15q24 segment either alone or in combination with other genetic abnormalities in hematopoietic cells. Further hematological investigation is recommended to be beneficial if physical and hematological examination results are suggestive of hematopoietic disturbance in patients with the syndrome.

Key words: 15q24 deletion syndrome; thrombocytopenia; neutropenia; myelodysplastic syndrome

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INTRODUCTION

Chromosome 15q24 deletion syndrome [OMIM#613406] is a recently-described disorder characterized by developmental delay, growth deficiency, distinct facial features, digital abnormalities, loose connective tissue, and genital malformations in males [Sharp et al., 2007]. Additional features include diaphragmatic hernia, bowel atresia, and congenital heart defects. The syndrome results from the interstitial deletion of the long arm of chromosome 15, typically detected by array comparative genomic hybridization (array CGH). To date, 19 patients with the syndrome have been reported [Smith et al., 2000; Sharp et al., 2007; Klopocki et al., 2008; Marshall et al., 2008; Andrieux et al., 2009; El-Hattab et al., 2010; Masurel-Paulet et al., 2009; Van Esch et al., 2009; El-Hattab et al., 2010; McInnes et al., 2010]. The patients shared some major clinical features.

Although El-Hattab et al. [2009] described a boy with the syndrome showing acute lymphoblastic leukemia (ALL), the relationship between hematological abnormalities and 15q24 deletion syndrome has not been reviewed. Here, we report on a boy with the syndrome manifesting myelodysplastic syndrome (MDS). MDS is a clonal disorder characterized by ineffective hematopoiesis, frequently progress to acute myeloid leukemia [Niemeyer et al., 2005]. We speculate on the mechanism of hematological abnormality in the syndrome.

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CLINICAL REPORTS

The patient, now a 13-year-old Japanese boy, was born as the second child of healthy nonconsanguineous parents. His mother had an operation for cervical incompetency during pregnancy. At 39 weeks of gestation, he was born by spontaneous delivery. His birth weight was 2,600 g (5th centile), length 49.5 cm (50th centile), and occipital frontal circumference (OFC) 33 cm (50th centile). Hypospadias and malformed ears were noted at birth.

At age 5 months, he presented with recurrent episodes of fever. Laboratory investigations revealed thrombocytopenia (platelet count, $36 \times 10^3/\mu l$). Bone marrow examination showed normal cellular elements with increasing megakaryocytes. The serum level of platelet associated immunoglobulin G was elevated at 160.0 ng/10^7 cells (normal range, 5–25 ng/10^7 cells). His condition was diagnosed as idiopathic thrombocytopenic purpura. The administration of corticosteroid and immunoglobulin infusion improved thrombocytopenia temporarily. However, thrombocytopenia occurred again at age 10 months and persisted up to the present time with occasionally subcutaneous hemorrhage (platelet count between 70 and $100 \times 10^3/\mu l$). He had recurrent respiratory infections with frequent admission in his childhood and school age. At age 10 years, he was first noted as having moderate neutropenia (absolute neutrophil count, $0.84 \times 10^3/\mu l$).

Developmental retardation was evident at age 5 months. He sat unsupported at 12 months, walked at 25 months, and spoke his first word at 24 months. A G-banding chromosomal analysis showed a normal karyotype. Metabolic investigations, cardiac ultrasonography, cranial magnetic resonance imaging, and electroencephalography all obtained normal findings. An ophthalmologic investigation showed mild myopia. An otological examination showed severe bilateral mixed hearing impairment. The Wechsler Intelligence Scale for Children at age 7 years showed mild intellectual disability.

When seen by us at age 13 years, his height was 149.5 cm (10th–25th centile), weight 37.6 kg (10th–25th centile), and OFC 55 cm (50th–75th centile). He had a long face, broad medial eyebrows, epicanthal folds, downslanting palpebral fissures, a flat nasal bridge, a high palate, a smooth philtrum, a full lower lip, cupped ears, short fifth fingers, hypospadias, mild digital joint contractures, and mild scoliosis (Fig. 1). He also showed severe

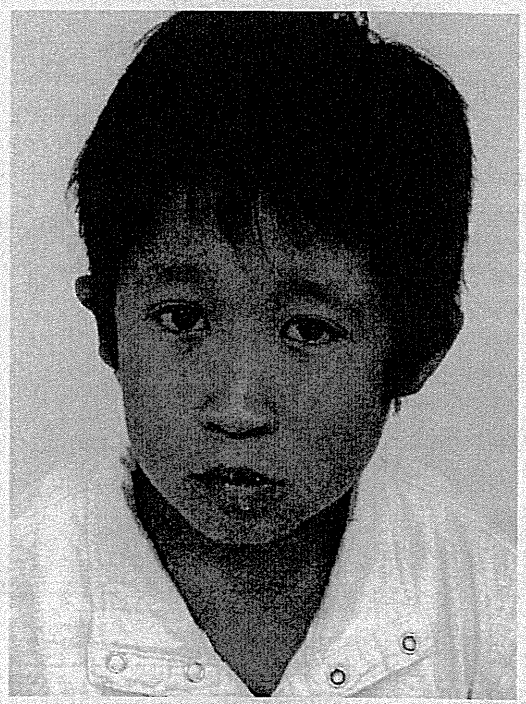

FIG. 1. A photogram of the patient at age 13-year old. A long face, broad medial eyebrows, epicanthal folds, down-slanting palpebral fissures, cupped ears, a flat nasal bridge, a smooth philtrum, and a full lower lip.

obstructive sleep apnea attributable to tonsillar hypertrophy. Hepatosplenomegaly was not observed. Though the hemoglobin level was normal at 13.3 g/dl, total white blood cell count was decreased at $1.9 \times 10^3/\mu l$, absolute neutrophil count was decreased at $0.43 \times 10^3/\mu l$, and platelet count was decreased at $68 \times 10^3/\mu l$. Serum levels of coagulation factors were all normal range. The serum immunoglobulin levels were normal. The results of the autoimmune screening were positive for anti-cardiolipin antibody (17 U/ml; normal range <10 U/ml) and negative for anti-double-stranded DNA antibody, antinuclear antibodies, anti-smith

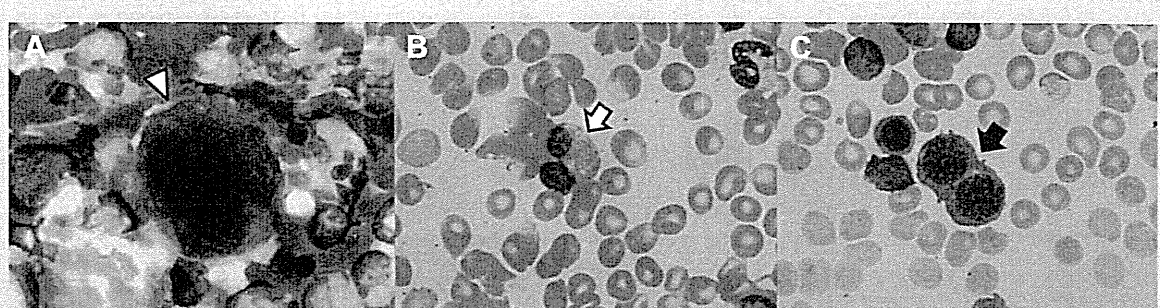

FIG. 2. Bone marrow findings of the present patient. A: Small megakaryocyte is noticeable (white arrow head). B: Pseudo-Pelger abnormality in neutrophil is shown (white arrow). C: Bilobed nuclei in erythroblast is noted (black arrow).

antibody, rheumatoid factor, antiplatelet antibody, platelet-associated immunoglobulin G, and antineutrophil antibody. Serological and polymerase chain reaction testing for parvovirus B19, cytomegalovirus, and Ebstein–Barr virus did not show an evidence of active infection. Chromosomal breakage with mitomycin C in peripheral blood lymphocytes was not increased. Monosomy 7 in bone marrow cells and telomere shortening in peripheral blood cells were not observed by cytogenetic analysis.

A bone marrow examination showed mild trilineage dysplasia with normal cellularity and increased number of megakaryocytes. Blast cell count was three percent. Megakaryocytes with single nuclei and noticeable small size, neutrophils with poor granulation and pseudo-Pelger abnormalities, and erythroblasts with binuclearity and mild hypoplasia were noted (Fig. 2A–C). According to the criteria by the fourth edition of the World Health Organization classification of hematopoietic and lymphoid neoplasms [Baumann et al., 2008], his condition was classified as childhood MDS.

MOLECULAR CYTOGENETIC INVESTIGATIONS

The study was conducted according to the Declaration of Helsinki and was approved by the Ethics Committee of Shinshu University School of Medicine, and informed consent was obtained from the parents of the patient.

DNA was extracted using the Gentra Puregene Blood Kit (Qiagen, Inc., Valencia, CA) according to the manufacturer's instructions. Array CGH was performed using the CGX-3 cytogenetics arrays (Roche NimbleGen, Inc., Madison, WI). This platform included 134,829 oligonucleotide probes covering the whole genome at an average resolution of 35 kb as well as clinically significant regions at 10 kb. The procedures for DNA labeling, and hybridization

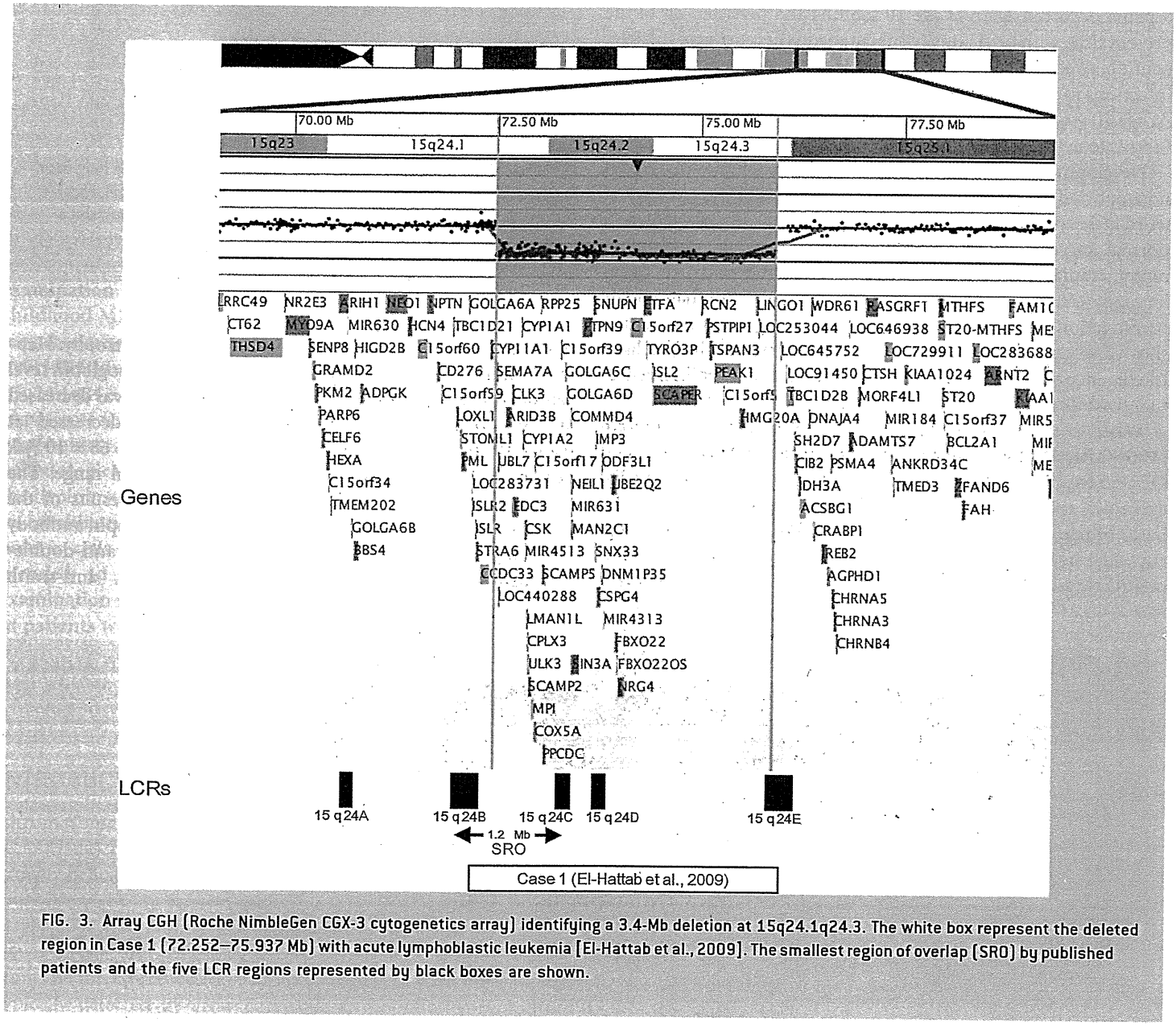

FIG. 3. Array CGH (Roche NimbleGen CGX-3 cytogenetics array) identifying a 3.4-Mb deletion at 15q24.1q24.3. The white box represent the deleted region in Case 1 (72.252–75.937 Mb) with acute lymphoblastic leukemia [El-Hattab et al., 2009]. The smallest region of overlap (SRO) by published patients and the five LCR regions represented by black boxes are shown.

were performed according to the manufacturer's instructions. The slides were scanned into image files using a NimbleGen MS 200 scanner. The array data analysis was performed using Genoglyphix Software (Signature Genomics Laboratories, Spokane, WA). Mapping data were analyzed on the Genoglyphix Genome Browser. All genomic locations correspond to NCBI build 36 (hg18). The array CGH analysis of the patient showed a deletion at chromosome region 15q24.1–15q24.3. The minimum size of the deletion was estimated as 3.44 Mb from the probe at 15q24.1 (chr15: 72,485,279 bp) to the probe at 15q24.3 (chr15: 75,921,984 bp) (Fig. 3).

To confirm array CGH findings, fluorescence in situ hybridization (FISH) analysis was performed on peripheral blood lymphocytes of the patient. BAC clones RP11-195A1 and RP11-91J9 were deleted and permitted to confirm the interstitial deletion on chromosome 15q24.1–q24.3 (data not shown). G-banding chromosomes of the parents were normal. The FISH analysis using two deleted BAC clones detected both two signals on the parental chromosomes. Thus, the aberration observed in present patient occurred de novo.

DISCUSSION

We report on a 13-year-old boy with characteristic facial features, hypospadias, mild developmental delay manifesting MDS. Array CGH demonstrated 15q24 deletion. This syndrome was recently delineated by Sharp et al. [2007]. Several patients had been described as having deletions encompassing the 15q24 segment, examined by standard chromosomal analysis and FISH studies [Cushman et al., 2005]. They showed many overlapping clinical features with the 15q24 deletion syndrome. However, their breakpoints had not been scrutinized. Most patients with 15q24 deletion syndrome are reported to have recurrent breakpoints, which are supposed to be mediated by nonallelic homologous recombination (NAHR) between the five low-copy repeats (LCRs) region. The deletions ranged from 1.7 to 6.1 Mb, with the smallest region of overlap (SRO) spanning approximately 1.2 Mb (chr15: 72.1–73.3 Mb) [El-Hattab et al., 2010]. Present patient had a deleted region spanning approximately 3.4 Mb, which comprises 50 genes and include the previously delineated SRO (Fig. 3).

To date, 20 patients with 15q24 deletion syndrome, including present patient, have been reported. These patients shared major clinical manifestations, including developmental delay, growth deficiency, characteristic facial dysmorphism, digital abnormalities, loose connective tissue, and genital malformations in males. The patient showed most of the major features reported in previous patients, but did not show growth deficiency, loose connective tissue, or brain anomaly (Table I). The patient manifested MDS, which has never been described in patients with the syndrome. ALL has been described in a boy reported by El-Hattab et al. [2009], whose breakpoints were similar to those of present patient (Fig. 3).

Childhood MDS is a relatively rare and complex disease. It is difficult to diagnose childhood MDS when the blast count is not elevated and clonality cannot be established. Hence, children with myelodysplasia or suspected MDS must be extensively worked up for secondary causes of dyspoiesis including nutritional deficiency, medications, toxins, metabolic diseases, infections, autoimmune diseases, growth factor therapy, and congenital disorders of hematopoiesis. Among them, congenital disorders of hematopoiesis including Fanconi anemia, dyskeratosis congenita, Diamond–Blackfan syndrome, Down syndrome, and mitochondria cytopathy is responsible for 29–44% of pediatric patients in whom MDS develops [McKenna, 2004; Yin et al., 2010]. The examinations on the present patient had no findings to suggest these secondary causes. The bone marrow examination of the patient showed mild trilineage dysplasia. His condition was classified as childhood MDS [Baumann et al., 2008].

MDS progress rapidly to leukemia or slowly over many years. Patients have a deteriorating course with 30% evolving into acute

TABLE I. Clinical Features in the Present Patient Compared to 19 Published Patients With 15q24 Deletion Syndrome

	Present patient	Nineteen previously reported cases
Deletion length	3.44 Mb	1.7–6.1 Mb
	72.48–75.92	67.8–76.08
Inheritance	De novo	17 de novo, 2 unknown
Gender	Male	17 male, 2 female
Age	13y	5mo–33y
Growth retardation	–	5/19
Intellectual disability/ developmental delay	+	19/19
Facial abnormalities		
High forehead/anterior hair line	–	12/19
Long/narrow face	+	5/19
Broad medial eyebrows	+	7/19
Epicanthus folds	+	8/19
Hypertelorism	–	8/19
Downslanting palpebral fissures	+	8/19
Smooth/long philtrum	+	10/19
Full lower lip	+	7/19
Eye abnormalities		
Strabismus	–	7/19
Dysopia	+	3/19
Ear abnormalities		
Hearing impairment	+	4/19
Malformed ear	+	12/19
Brain malformation	–	9/19
Cardiac abnormalities	–	5/19
Urogenital abnormalities	+	11/19
Skeletal malformation		
Joint laxity	–	8/19
scoliosis	+	6/19
Digital abnormalities	+	14/19
Diaphragmatic hernia	–	3/19
Inguinal hernia	–	4/19
Gastrointestinal abnormalities	–	3/19
Recurrent infection	+	7/19
Hematopoietic disorder	+	1/19
Autism spectrum	–	3/19

mo, month; y, year.

leukemia usually of myeloid phenotype. Evolution into ALL from MDS is rare and seen in <1% adult cases and extremely rare in pediatric population. However, 26 patients with MDS progress to ALL were reported [Gupta and Bhatia, 2010]. El-Hattab et al. [2009] reported a boy with 15q24 deletion and ALL. They hypothesized some tumor-associated genes which located in 15q24 region, *C-Src* tyrosine kinase (*CSK*) and *SIN3A* may lead to increased risk of developing neoplasm. *UBL7/BMSC-UbP* located at 15q24.1 isolated from the bone marrow stromal cell cDNA library encodes a bone marrow stromal cell-derived ubiquitin-like protein. *UBL7* was suggested to a play role in the regulation of bone marrow stromal cell function or cell differentiation through an evocator and cell specific pattern [Liu et al., 2003]. The development of MDS and hematological malignancy in the syndrome might be caused by the haploinsufficiency of deleted 15q24 segment either alone or in combination with other genetic abnormalities in hematopoietic cells.

In conclusion, we report a patient with MDS and 15q24 deletion syndrome. This syndrome might be prone to have hematological malignancy. A careful hematological follow-up of the present patient is required. Further hematological investigation is recommended to be beneficial if physical and hematological examination results are suggestive of hematopoietic disturbance in patients with 15q24 deletion syndrome.

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ORIGINAL ARTICLE

Clinical application of array-based comparative genomic hybridization by two-stage screening for 536 patients with mental retardation and multiple congenital anomalies

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Recent advances in the analysis of patients with congenital abnormalities using array-based comparative genome hybridization (aCGH) have uncovered two types of genomic copy-number variants (CNVs); pathogenic CNVs (pCNVs) relevant to congenital disorders and benign CNVs observed also in healthy populations, complicating the screening of disease-associated alterations by aCGH. To apply the aCGH technique to the diagnosis as well as investigation of multiple congenital anomalies and mental retardation (MCA/MR), we constructed a consortium with 23 medical institutes and hospitals in Japan, and recruited 536 patients with clinically uncharacterized MCA/MR, whose karyotypes were normal according to conventional cytogenetics, for two-stage screening using two types of bacterial artificial chromosome-based microarray. The first screening using a targeted array detected pCNV in 54 of 536 cases (10.1%), whereas the second screening of the 349 cases negative in the first screening using a genome-wide high-density array at intervals of approximately 0.7 Mb detected pCNVs in 48 cases (13.8%), including pCNVs relevant to recently established microdeletion or microduplication syndromes, CNVs containing pathogenic genes and recurrent CNVs containing the same region among different patients. The results show the efficient application of aCGH in the clinical setting.
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Keywords: array-CGH; congenital anomaly; mental retardation; screening

INTRODUCTION

Mental retardation (MR) or developmental delay is estimated to affect 2–3% of the population.[1] However, in a significant proportion of cases, the etiology remains uncertain. Hunter[2] reviewed 411 clinical cases of MR and reported that a specific genetic/syndrome diagnosis was carried out in 19.9% of them. Patients with MR often have congenital anomalies, and more than three minor anomalies can be useful in the diagnosis of syndromic MR.[2,3] Although chromosomal aberrations are well-known causes of MR, their frequency determined by conventional karyotyping has been reported to range from 7.9 to 36% in patients with MR.[4–8] Although the diagnostic yield depends on the population of each study or clinical conditions, such studies

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suggest that at least three quarters of patients with MR are undiagnosed by clinical dysmorphic features and karyotyping.

In the past two decades, a number of rapidly developed cytogenetic and molecular approaches have been applied to the screening or diagnosis of various congenital disorders including MR, congenital anomalies, recurrent abortion and cancer pathogenesis. Among them, array-based comparative genome hybridization (aCGH) is used to detect copy-number changes rapidly in a genome-wide manner and with high resolution. The target and resolution of aCGH depend on the type and/or design of mounted probes, and many types of microarray have been used for the screening of patients with MR and other congenital disorders: bacterial artificial chromosome (BAC)-based arrays covering whole genomes,[9,10] BAC arrays covering chromosome X,[11,12] a BAC array covering all subtelomeric regions,[13] oligonucleotide arrays covering whole genomes,[14,15] an oligonucleotide array for clinical diagnosis[16] and a single nucleotide polymorphism array covering the whole genome.[17] Because genome-wide aCGH has led to an appreciation of widespread copy-number variants (CNVs) not only in affected patients but also in healthy populations,[18–20] clinical cytogenetists need to discriminate between CNVs likely to be pathogenic (pathogenic CNVs, pCNVs) and CNVs less likely to be relevant to a patient's clinical phenotypes (benign CNVs, bCNVs).[21] The detection of more CNVs along with higher-resolution microarrays needs more chances to assess detected CNVs, resulting in more confusion in a clinical setting.

We have applied aCGH to the diagnosis and investigation of patients with multiple congenital anomalies and MR (MCA/MR) of unknown etiology. We constructed a consortium with 23 medical institutes and hospitals in Japan, and recruited 536 clinically uncharacterized patients with a normal karyotype in conventional cytogenetic tests. Two-stage screening of copy-number changes was performed using two types of BAC-based microarray. The first screening was performed by a targeted array and the second screening was performed by an array covering the whole genome. In this study, we diagnosed well-known genomic disorders effectively in the first screening, assessed the pathogenicity of detected CNVs to investigate an etiology in the second screening and discussed the clinical significance of aCGH in the screening of congenital disorders.

MATERIALS AND METHODS

Subjects

We constructed a consortium of 23 medical institutes and hospitals in Japan, and recruited 536 Japanese patients with MCA/MR of unknown etiology from July 2005 to January 2010. All the patients were physically examined by an expert in medical genetics or a dysmorphologist. All showed a normal karyotype by conventional approximately 400–550 bands-level G-banding karyotyping. Genomic DNA and metaphase chromosomes were prepared from peripheral blood lymphocytes using standard methods. Genomic DNA from a lymphoblastoid cell line of one healthy man and one healthy woman were used as a normal control for male and female cases, respectively. All samples were obtained with prior written informed consent from the parents and approval by the local ethics committee and all the institutions involved in this project. For subjects in whom CNV was detected in the first or second screening, we tried to analyze their parents as many as possible using aCGH or fluorescence *in situ* hybridization (FISH).

Array-CGH analysis

Among our recently constructed in-house BAC-based arrays,[22] we used two arrays for this two-stage survey. In the first screening we applied a targeting array, 'MCG Genome Disorder Array' (GDA). Initially GDA version 2, which contains 550 BACs corresponding to subtelomeric regions of all chromosomes except 13p, 14p, 15p, 21p and 22p and causative regions of about 30 diseases already reported, was applied for 396 cases and then GDA version 3, which contains 660 BACs corresponding to those of GDA version 2 and pericentromeric regions of all chromosomes, was applied for 140 cases. This means that a CNV detected by GDA is certainly relevant to the patient's phenotypes. Subsequently in the second screening we applied 'MCG Whole Genome Array-4500' (WGA-4500) that covers all 24 human chromosomes with 4523 BACs at intervals of approximately 0.7 Mb to analyze subjects in whom no CNV was detected in the first screening. WGA-4500 contains no BACs spotted on GDA. If necessary, we also used 'MCG X-tiling array' (X-array) containing 1001 BAC/PACs throughout X chromosome other than pseudoautosomal regions.[12] The array-CGH analysis was performed as previously described.[12,23]

For several subjects we applied an oligonucleotide array (Agilent Human Genome CGH Microarray 244K; Agilent Technologies, Santa Clara, CA, USA) to confirm the boundaries of CNV identified by our in-house BAC arrays. DNA labeling, hybridization and washing of the array were performed according to the directions provided by the manufacturer. The hybridized arrays were scanned using an Agilent scanner (G2565BA), and the CGH Analytics program version 3.4.40 (Agilent Technologies) was used to analyze copy-number alterations after data extraction, filtering and normalization by Feature Extraction software (Agilent Technologies).

Fluorescence *in situ* hybridization

Fluorescence *in situ* hybridization was performed as described elsewhere[23] using BACs located around the region of interest as probes.

RESULTS

CNVs detected in the first screening

In the first screening, of 536 cases subjected to our GDA analysis, 54 (10.1%) were determined to have CNV (Figure 1; Tables 1 and 2).

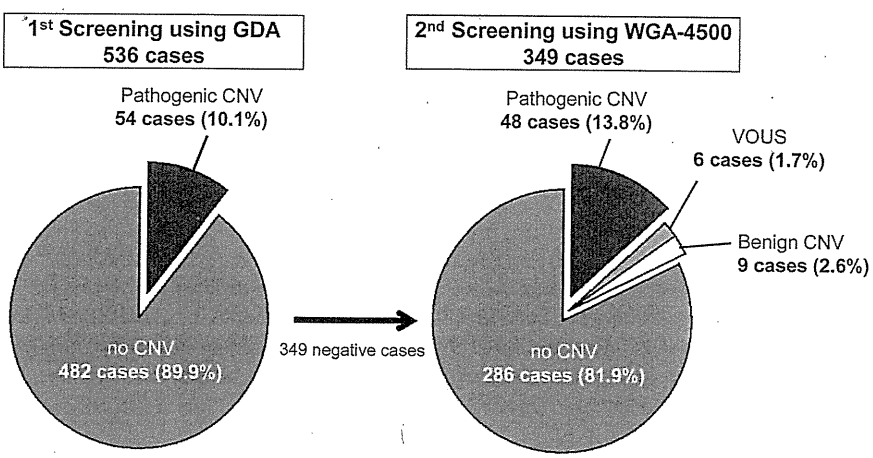

Figure 1 Percentages of each screening in the current study.

Table 1 A total of 40 cases with CNV at subtelomeric region(s) among 54 positive cases in the first screening

	Position where CNV detected				
Gender	*Loss*	*Gain*	*Corresponding disorder*[a]	*OMIM or citation*	*Parental analysis*[b]
M	1p36.33		Chromosome 1p36 deletion syndrome	#607872	
M	1p36.33p36.32		Chromosome 1p36 deletion syndrome	#607872	
M	1p36.33p36.32		Chromosome 1p36 deletion syndrome	#607872	
M	1p36.33p36.32		Chromosome 1p36 deletion syndrome	#607872	
M	1q44		Chromosome 1q43-q44 deletion syndrome	#612337	
F	2q37.3		2q37 monosomy[c]	Shrimpton *et al.*[24]	
F	2q37.3		2q37 monosomy[c]	Shrimpton *et al.*[24]	
M	3q29		Chromosome 3q29 deletion syndrome	#609425	
F	5p15.33p15.32		Cri-du-chat syndrome	#123450	
M	5q35.2q35.3		Chromosome 5q subtelomeric deletion syndrome	Rauch *et al.*[25]	
F	6p25.3		Chromosome 6pter-p24 deletion syndrome	#612582	
M	7q36.3		7q36 deletion syndrome[d]	Horn *et al.*[26]	
F	7q36.3		7q36 deletion syndrome[d]	Horn *et al.*[26]	
M	9p24.3p24.2		Chromosome 9p deletion syndrome	#158170	
F	9q34.3		Kleefstra syndrome	#610253	
F	10q26.3		Chromosome 10q26 deletion syndrome	#609625	
F	16p13.3		Chromosome 16p13.3 deletion syndrome	#610543	
F	22q13.31		Chromosome 22q13 deletion syndrome	#606232	
M	22q13.31q13.33		Chromosome 22q13 deletion syndrome	#606232	
M		15q26.3	15q overgrowth syndrome[c]	Tatton-Brown *et al.*[27]	
F		15q26.3	15q overgrowth syndrome[c]	Tatton-Brown *et al.*[27]	
M		21q22.13q22.3	Down's syndrome (partial trisomy 21)	#190685	
M		Xp22.33	A few cases have been reported; e.g. V5-130 in Lu *et al.*[28]		
M		Xq28	Chromosome Xq28 duplication syndrome	#300815	
F	1q44		Chromosome 1q43-q44 deletion syndrome	#612337	
		8p23.2p23.3			
M	3p26.3		3p deletion syndrome[d]	Fernandez *et al.*[29]	
		12p13.33p11.22			
F	3p26.3		3p deletion syndrome[d]	Fernandez *et al.*[29]	
		16p13.3	Chromosome 16p13.3 duplication syndrome	#613458	
F	4q35.2		4q– syndrome[d]	Jones *et al.*[30]	
		7q36.3			
M	5p15.33		Cri-du-chat syndrome	#123450	
		20p13			
M	5p15.33p15.32		Cri-du-chat syndrome	#123450	
		2p25.3			
F	6q27		6q terminal deletion syndrome[d]	Striano *et al.*[31]	
		11q25			
F	6q27		6q terminal deletion syndrome[d]	Striano *et al.*[31]	
		8q24.3			
M	7q36.3		7q36 deletion syndrome[d]	Horn *et al.*[26]	*dn*
		1q44			
M	9p24.3p24.2		Chromosome 9p deletion syndrome	#158170	
		7q36.3			
F	10p15.3p15.2		Chromosome 10p terminal deletion[d]	Lindstrand *et al.*[32]	*pat*
		7p22.3p22.2			
M	10p15.3		Chromosome 10p terminal deletion[d]	Lindstrand *et al.*[32]	
		2p25.3			
M	10q26.3		Chromosome 10q26 deletion syndrome	#609625	
		2q37.3	Distal trisomy 2q[d]	Elbracht *et al.*[33]	
M	18q23		Chromosome 18q deletion syndrome	#601808	
		7q36.3			
F	22q13.31q13.33		Chromosome 22q13.3 deletion syndrome	#606232	*pat*
		17q25.3	One case was reported	Lukusa *et al.*[34]	
M	Xp22.33/Yp11.32		Contiguous gene–deletion syndrome on Xp22.3[d]	Fukami *et al.*[35]	
		Xq27.3q28	Chromosome Xq28 duplication syndrome	#300815	

Abbreviations: F, female; CNV, copy-number variant; M, male; OMIM, Online Mendelian Inheritance in Man; *dn*, *de novo* CNV observed in neither of the parents.
[a]The name of disorder is based on entry names of OMIM, expect for entry names in DECIPHER and description in each cited article.
[b]*pat*, father had a balanced translocation involved in corresponding subtelomeric regions.
[c]Entry names in DECIPHER.
[d]Description in each cited article.

All the CNVs detected in the first screening were confirmed by FISH. Among the positive cases, in 24 cases one CNV was detected. All the CNVs corresponded to well-established syndromes or already described disorders (Table 1). In 16 cases two CNVs, one deletion and one duplication, were detected at two subtelomeric regions, indicating that one of parents might be a carrier with reciprocal translocation involved in corresponding subtelomeric regions, and at least either of the two CNVs corresponded to the disorders. We also performed parental analysis by FISH for three cases whose parental samples were available, and confirmed that in two cases the subtelomeric aberrations were inherited from paternal balanced translocation and in one case the subtelomeric aberrations were *de novo* (Table 1). In the other 14 cases, CNVs (25.9%) were detected in regions corresponding to known disorders (Table 2).

Table 2 Other cases among 54 positive cases in the first screening

	Position where CNV detected			
Gender	*Gain*	*Loss*	*Corresponding disorder*	*OMIM*
F		4p16.3	Ring chromosome	
		4q35.2		
M		3q22.323	BPES	#110100
M		2q22.3	ZFHX1B region	*605802
M		4q22.1	Synuclein (SNCA) region	*163890
F		7p21.1	Craniosynostosis, type 1	#123100
F		7q11.23	Williams syndrome	#194050
F		8q23.3q24.11	Langer–Giedion syndrome	#150230
M	15q11.2q13.1		Prader–Willi/Angelman	#176270/ #105830
F		17p11.2	Smith–Magenis syndrome	#182290
M		17q11.2	Neurofibromatosis, type I	+162200
M	22q11.21		DiGeorge syndrome	#188400
F		22q11.21	DiGeorge syndrome	#188400
F	Xp22.31		Kallmann syndrome 1	+308700
F	Whole X		Mosaicism	

Abbreviations: CNV, copy-number variant; F, female; M, male; OMIM, Online Mendelian Inheritance in Man.

CNVs detected in the second screening and assessment of the CNVs

Cases were subject to the second screening in the order of subjects detected no CNV in the first screening, and until now we have analyzed 349 of 482 negative cases in the first screening. In advance, we excluded highly frequent CNVs observed in healthy individuals and/or in multiple patients showing disparate phenotypes from the present results based on an internal database, which contained all results of aCGH analysis we have performed using WGA-4500, or other available online databases; for example, Database of Genomic Variant (http://projects.tcag.ca/variation/). As a result, we detected 66 CNVs in 63 cases (Figure 1; Table 3). Among them, three patients (cases 36, 42 and 44) showed two CNVs. All the CNVs detected in the second screening were confirmed by other cytogenetic methods including FISH and/or X-array. For 60 cases, we performed FISH for confirmation and to determine the size of each CNV. For five cases, cases 13, 36, 48, 57 and 63, with CNVs on the X chromosome, we used the X-array instead of FISH. For cases 4, 6, 16–19 and 34, we also used Agilent Human Genome CGH Microarray 244K to determine the refined sizes of CNVs. The maximum and minimum sizes of each CNV determined by these analyses are described in Table 3.

Well-documented pCNVs emerged in the second screening

CNVs identified for recently established syndromes. We assessed the pathogenicity of the detected CNVs in several aspects (Figure 2).[21,37,38] First, in nine cases, we identified well-documented pCNVs, which are responsible for syndromes recently established. A heterozygous deletion at 1q41–q42.11 in case 2 was identical to patients in the first report of 1q41q42 microdeletion syndrome.[39] Likewise a CNV in case 3 was identical to chromosome 1q43–q44 deletion syndrome (OMIM: #612337),[40] a CNV in case 4 was identical to 2q23.1 microdeletion syndrome,[41] a CNV in case 5 was identical to 14q12 microdeletion syndrome[42] and a CNV in case 6 was identical to chromosome 15q26-qter deletion syndrome (Drayer's syndrome) (OMIM: #612626).[43] Cases 7, 8 and 9 involved CNVs of different sizes at 16p12.1-p11.2, the region responsible for 16p11.2-p12.2 microdeletion syndrome.[44,45] Although an interstitial deletion at 1p36.23-p36.22 observed in case 1 partially overlapped with a causative region of chromosome 1p36 deletion syndrome (OMIM: #607872), the region deleted was identical to a proximal interstitial 1p36 deletion that was recently reported.[46] Because patients with the proximal 1p36 deletion including case 1 demonstrated different clinical characteristics from cases of typical chromosome 1p36 deletion syndrome, in the near term their clinical features should be redefined as an independent syndrome.[46]

CNVs containing pathogenic gene(s). In four cases we identified pCNVs that contained a gene(s) probably responsible for phenotypes. In case 10, the CNV had a deletion harboring *GLI3* (OMIM: *165240) accounting for Greig cephalopolysyndactyly syndrome (GCS; OMIM: 175700).[47] Although phenotypes of the patient, for example, pre-axial polydactyly of the hands and feet, were consistent with GCS, his severe and atypical features of GCS, for example, MR or microcephaly, might be affected by other contiguous genes contained in the deletion.[48] Heterozygous deletions of *BMP4* (OMIM: *112262) in case 11 and *CASK* (OMIM: *300172) in case 13 have been reported previously.[49,50] In case 12, the CNV contained *YWHAE* (OMIM: *605066) whose haploinsufficiency would be involved in MR and mild CNS dysmorphology of the patient because a previous report demonstrated that haploinsufficiency of *ywhae* caused a defect of neuronal migration in mice[51] and a recent report also described a microdeletion of *YWHAE* in a patient with brain malformation.[52]

Recurrent CNVs in the same regions. We also considered recurrent CNVs in the same region as pathogenic; three pairs of patients had overlapping CNVs, which have never been reported previously. Case 16 had a 3.3-Mb heterozygous deletion at 10q24.31–q25.1 and case 17 had a 2.0-Mb deletion at 10q24.32–q25.1. The clinical and genetic information will be reported elsewhere. Likewise, cases 14 and 15 also had an overlapping CNV at 6q12–q14.1 and 6q14.1, and cases 18 and 19 had an overlapping CNV at 10p12.1–p11.23. Hereafter, more additional cases with the recurrent CNV would assist in defining new syndromes.

CNVs reported as pathogenic in previous studies. Five cases were applicable to these criteria. A deletion at 3p21.2 in case 20 overlapped with that in one case recently reported.[53] The following four cases had CNVs reported as pathogenic in recent studies: a CNV at 7p22.1 in case 21 overlapped with that of patient 6545 in a study by Friedman *et al.*,[14] a CNV at 14q11.2 in case 22 overlapped with those of patients 8326 and 5566 in Friedman *et al.*,[14] a CNV at 17q24.1–q24.2 in case 23 overlapped with that in patient 99 in Buysse *et al.*[54] and a CNV at 19p13.2 in case 24 overlapped with case P11 in Fan *et al.*[55]

Large or gene-rich CNVs, or CNVs containing morbid OMIM genes. In cases inapplicable to the above criteria, we assessed CNVs

Table 3 Sixty-three cases with CNV in the 2nd screening

Case	Gender	Clinical diagnosis	Remarkable clinical features	CNV	Position	WGA-4500[b]	FISH[b]	Base position and size of the identified CNV[a]						Parental analysis	Protein-coding genes[c]	CNV assessment[d]	Corresponding or candidate gene(s)
								Start (max)	Start (min)	End (min)	End (max)	Size (min)	Size (max)				
1	M	MCA/MR		del	1p36.23p36.22	arr cgh 1p36.23p36.22 (RP11-81J7→RP11-199O1)x1	ish del(1)(p36.23p36.22) (RP11-462M3+, RP11-106A3-, RP11-28P4+)dn	8 585 127	8 890 860	10 561 097	11 143 717	1 670 237	2 558 590	*dn*	32	P	
2	M	MCA/MR		del	1q41q42.11	arr cgh 1q41 (RP11-135J2→RP11-239E10)x1	ish del(1)(q41q42.11) (RP11-706L9+, RP11-224O19-, RP11-367O4-)dn	215 986 492	216 532 600	221 534 398	222 467 931	5 001 798	6 481 439	*dn*	35	P	
3	F	MCA/MR	Epilepsy	del	1q44	arr cgh 1q44 (RP11-156E8)x1	ish del(1)(q44) (RP11-56O19+, RP11-156E8-)	241 996 973	243 177 632	243 251 660	244 141 010	74 028	2 144 037		11	P	
4	F	MCA/MR		del	2q22	arr cgh 2q23.1 (RP11-72H23)x1	ish del(2)(q23.1) (RP11-375H16-)	147 651 472	147 688 255	149 855 826	149 879 891	2 167 571	2 228 419		7	P	
5	F	MCA/MR		del	14q12q13.2	arr cgh 14q12q13.2 (RP11-369O9→RP11-26M6)x1	ish del(14)(q13.2) (RP11-831F6-)	28 768 137	29 297 829	34 689 412	35 489 337	5 391 583	6 721 200		25	P	
6	M	MCA/MR	CHD	del	15q26.2	arr cgh 15q26.2q26.3 (RP11-79C10→RP11-80F4)x1	ish del(15)(q26.2) (RP11-308P12-)	93 199 415	93 214 053	96 928 421	96 942 334	3 714 368	3 742 919		6	P	
7	M	MCA/MR	CHD	del	16p12.1p11.2	arr cgh 16p12.1p11.2 (RP11-309I14→RP11-150K5)x1	ish del(16)(p11.2) (RP11-75J11-)dn	25 795 340	27 008 538	29 825 404	31 443 492	2 816 866	5 648 152	*dn*	138	P	
8	M	MCA/MR	CHD	del	16p11.2	arr cgh 16p12.1p11.2 (RP11-360L15→RP11-150K5)x1	ish del(16)(p11.2) (RP11-360L15-, RP11-388M20+, RP11-75J11+)dn	27 184 508	28 873 631	29 825 404	31 443 492	951 773	4 258 984	*dn*	134	P	
9	F	MCA/MR		del	16p11.2	arr cgh 16p11.2 (RP11-368N21→RP11-499D5)x1	ish del(16)(p11.2) (RP11-388M20-, RP11-75J11-)	28 873 841	29 408 698	32 773 200	34 476 095	3 364 502	5 602 254		125	P	
10	M	MCA/MR		del	7p14.2p13	arr cgh 7p14.2p13 (RP11-138E20→RP11-52M17)x1	ish del(7)(p14.1p13) (RP11-258I11+, RP11-2J17-, RP11-346F12-)dn	35 621 006	36 470 190	44 657 334	45 508 196	8 187 144	9 887 190	*dn*	70	P	*GLI3*
11	F	MCA/MR	Corneal opacity	del	14q22.1q22.3	arr cgh 14q22.1q22.3 (RP11-122A4→RP11-172G1)x1	ish del(14)(q22.1) (RP11-122A4-, RP11-316L15+)dn	51 964 774	51 983 834	54 730 496	55 054 754	2 746 662	3 089 980	*dn*	18	P	*BMP4*
12	M	MCA/MR	Idiopathic leukodystrophy	del	17q13.3	arr cgh 17p13.3 (RP11-294J5→RP11-357O7)x1	ish del(17)(p13.3) (RP11-4F24-, RP11-26N6+)dn	1 008 128	1 146 211	2 077 151	2 026 967	930 940	1 018 839	*dn*	22	P	*YWHAE*
13	M	MCA/MR		del	Xp11.4p11.3	arr cgh Xp11.3p11.4 (RP11-1069J5→RP11-245M24)x1	ish del(X)(p11.4p11.3) (RP11-95C16-, RP11-829C10-)dn	41 392 291	41 385 453	45 419 624	45 495 709	4 034 171	4 103 418	*dn*	9	P	*CASK*

Table 3 Continued

Case	Gender	Clinical diagnosis	Remarkable clinical features	CNV	Position	WGA-4500[b]	FISH[b]	Base position and size of the identified CNV[a]						Parental analysis	Protein-coding genes[c]	CNV assessment[d]	Corresponding or candidate gene(s)
								Start (max)	Start (min)	End (min)	End (max)	Size (min)	Size (max)				
14	M	MCA/MR		del	6q12q14.1	arr cgh 6q12q14.2(RP11-502L6→RP11-232L4)x1	ish del(6)(q13)(RP11-28P18-)dn	69 029 871	69 731 888	83 926 178	85 101 718	14 194 290	16 071 847	*dn*	56	P	
15	M	ZLS		del	6q14.1	arr cgh 6q14.1(RP11-343P23→RP11-217L13)x1	ish del(6)(q14.1)(RP11-5N7-,RP11-990K4-,RP11-1I6+)	75 484 004	76 145 436	79 474 428	79 851 528	3 328 992	4 367 524		10	P	
16	F	MCA/MR	CHD	del	10p12.1p11.23	arr cgh 10p12.1p11.23(RP11-89D1→91A23)x1	ish del(10)(p12.1p11.23)(RP11-164A7-,RP11-110B21-)	27 045 285	27 054 002	29 057 401	29 088 950	2 003 399	2 043 665		18	P	
17	M	MCA/MR		del	10p12.1p11.23	arr cgh 10p12.1p11.23(RP11-218D6→RP11-RP11-181I11)x1	ish del(10)(p11.23)(RP11-15H10-)	28 121 596	28 131 608	30 559 024	30 577 807	2 427 416	2 456 211		12	P	
18	M	MCA/MR	CHD	del	10q24.31q25.1	arr cgh 10q24.31q25.1(RP11-108L7→RP11-108L7)x1	ish del(10)(q24.33)(RP11-416N2-)dn	102 560 783	102 568 462	105 914 057	105 929 608	3 345 595	3 368 825	*dn*	66	P	
19	M	MCA/MR		del	10q24.32q25.1	arr cgh 10q24.32q25.1(RP11-21N23→RP11-99N20)x1	ish del(10)(q24.33)(RP11-416N2-)dn	103 917 900	103 928 189	106 005 827	106 011 522	2 077 638	2 093 622	*dn*	41	P	
20	F	MCA/MR		del	3p21.31p21.2	arr cgh 3p21.31p21.2(RP11-24F11→RP11-89F17)x1	ish del(3)(p21.31)(RP11-3B7-)	46 150 261	46 359 965	51 390 597	52 571 544	5 030 632	6 421 283		175	P	
21	M	MCA/MR		del	7p22.1	arr cgh 7p22.1(RP11-90J23→RP11-2K20)x1	ish del(7)(p22.1)(RP11-2K20-)dn	3 185 609	5 892 225	6 233 987	6 409 277	341 762	3 223 668	*dn*	28	P	
22	F	MCA/MR	Corneal opacity, CHD	dup	14q11.2	arr cgh 14q11.2(RP11-152G22→RP11-84D12)x3	ish dup(14)(q11.2)(RP11-152G22++)	20 070 731	20 306 624	20 534 929	21 264 945	228 305	1 194 214		>30	P	
23	M	MCA/MR		del	17q24.1q24.2	arr cgh 17q24.1q24.2(RP11-89L7→RP11-79K13)x1	ish del(17)(q24.1q24.2)(RP11-93E5-,RP11-89L7-,RP11-79K13-)	60 576 365	60 936 391	64 592 701	64 587 782	3 656 310	4 011 417		29	P	
24	M	SMS susp.		del	19p13.2	arr cgh 19p13.2(RP11-19704→RP11-164D24)x1	ish del(19)(p13.2)(91021-)	9 248 377	10 248 853	11 968 772	12 553 279	1 719 919	3 304 902	*dn*		P	
25	M	MCA/MR	Epilepsy	dup	2q11.2q13	arr cgh 2q11.2q13(RP11-90G13→RP11-79K7)x3	ish dup(2)(q11.2)(RP11-542D13++)	88 273 220	91 696 986	109 869 691	112 714 666	18 172 705	24 441 446		>30	P	
26	M	MCA/MR	CHD	dup	4p16.1	arr cgh 4p16.1(RP11-17I9)x3	ish dup(4)(p16.1)(RP11-301J10++)	8 202 790	8 520 479	9 793 705	10 638 054	1 273 226	2 435 264		17	P	