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Clinical Report

Tetralogy of Fallot Associated With Pulmonary Atresia and Major Aortopulmonary Collateral Arteries in a Patient With Interstitial Deletion of 16q21–q22.1

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A newborn male had an interstitial deletion of 16q21–q22.1 accompanying tetralogy of Fallot associated with pulmonary atresia and major aortopulmonary collateral arteries (MAPCA), dysmorphic craniofacial features, failure to thrive, and severe psychomotor developmental delay. When the deletion in this patient and other reported patients are compared, the 16q22 region appears to be the smallest region for 16q deletion syndrome. Since over 50% of patients with the deletion of 16q22 region have congenital heart disease, there may be a responsible gene in this region.

Key words: congenital heart disease; microdeletion; contiguous gene syndrome; malformation; chromosome 16; conotruncal heart defect

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INTRODUCTION

There are at least 20 reported patients with deletion of chromosome 16q in the literature: [Fryns et al., 1977, 1981; Taysi et al., 1978; Lin et al., 1983; Elder et al., 1984; Hoo et al., 1985; Rivera et al., 1985; Cooke et al., 1987; Krauss et al., 1987; Natt et al., 1987, 1989; Naritomi et al., 1988; Casamassima et al., 1990; Edelhoff et al., 1991; Fujiwara et al., 1992; Schuffenhauer et al., 1992; Callen et al., 1993; Doco-Fenzy et al., 1994; Khan et al., 2006]. These patients show some overlapped phenotypes, including small birth weight, postnatal growth delay, psychomotor delay, high forehead, flat nasal bridge, hypertelorism, and other visceral malformations. However, the identification of a critical region for this deletion syndrome has been ambiguous, with both 16q12–13 and 16q22.1 being suggested as critical [Callen et al., 1993].

Recently, we encountered a baby boy with interstitial deletion of 16q21–22.1. In addition to the common features of 16q deletion syndrome, he showed a very characteristic congenital heart disease (CHD), including major aortopulmonary collateral arteries (MAPCA). The etiology of his CHD and MAPCA will be discussed in this study.

CLINICAL REPORT

A woman was referred to our institution at 24 weeks of pregnancy because a fetal echocardiogram revealed CHD but without any details. The mother and

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her husband were healthy, and their first child, a girl, weighted 3,580 g at birth and was healthy.

A newborn male was born at 38 weeks and 4 days gestational age when the mother was 29 years old showing intrauterine growth retardation with a birth weight of 2,492 g (<10th centile), length of 45 cm (<10th centile), and head circumference of 30.8 cm (<3rd centile). A postnatal echocardiogram showed tetralogy of Fallot (TOF) associated with pulmonary atresia (PA) and MAPCA. He also showed multiple anomalies with dysmorphic craniofacial features (i.e. hypertelorism, epicanthic folds, upslanting palpebral fissures, bilateral cleft lip, and cleft palate) (Fig. 1A), bilateral simple palmar crease, overlapping fingers, and one neonatal tooth. Catheter angiography performed at 18 days of age confirmed a severely hypoplastic central pulmonary artery of less than 1 mm in diameter. After he was 2-months old, a right modified Blalock-Taussig shunt operation, unifocalization and interventional catheterization were performed, and catheter angiography at 5 months showed growth of the central pulmonary artery.

As he could not control his head until 10 months of age, his psychomotor development was moderately retarded and at the same age he could not turn over.

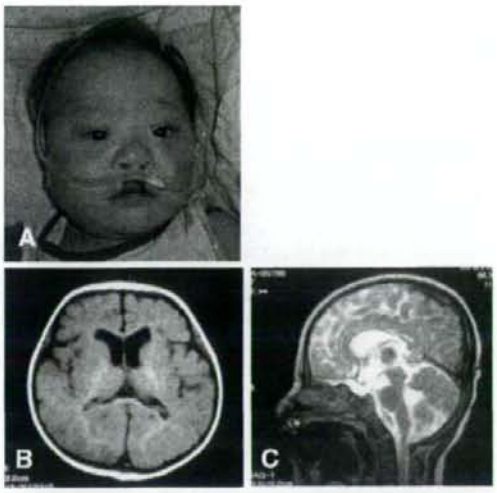

FIG. 1. Facial expression of the patient (**A**). Dysmorphic appearance with prominent forehead, broad nasal bridge, upslanting palpebral fissures, and cleft lip is seen in a photograph taken when he was 12 months of age. Brain magnetic resonance image at 12 months old showing an axial section (**B**) and saggital section (**C**). Mild delay of myelination, mild dilatation of the lateral ventricles and hypoplasia of the splenium of the corpus callosum are shown.

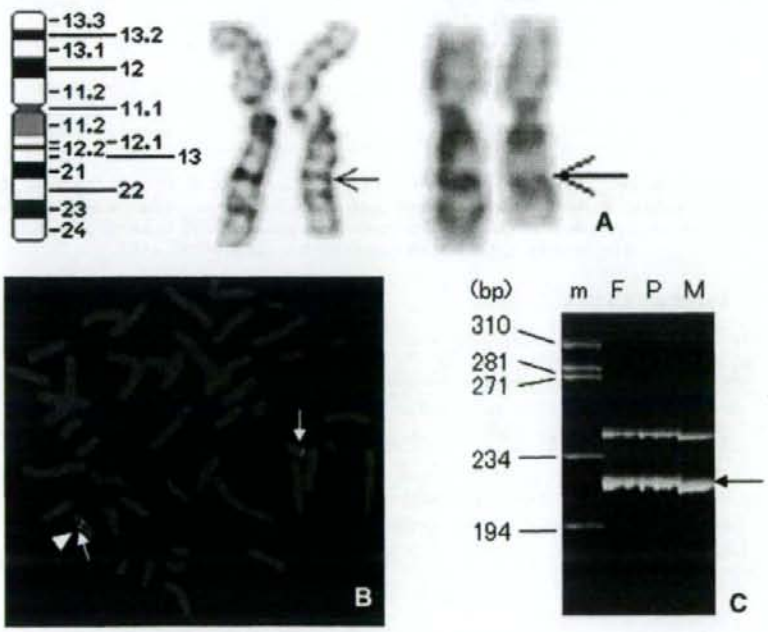

FIG. 2. G-banded chromosome 16 of proposita (**A**). Normal (left) and deleted 16q (right) are shown compared with a schematic representation of chromosome 16. Two-color FISH analysis determined the deletion of the 16q21–22.2 region (**B**). The orange (arrowhead) and green signal (arrows) indicate RP11-828P4 (16q21) and RP11-110A3 (16q21), respectively. Only one orange signal is seen and this indicates a deletion of this region. Examination of the parental origin of the deletion (**C**). Electrophoretic bands of PCR products for microsatellite marker, D16S3129, of the patient and his parents are visualized by ethidium bromide staining. The patient shows bands common to only the paternal allele, and the maternal allele is not common to the patient's band (arrow). m, marker (φX174/HaeIII digest); F, father; P, patient; M, mother. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

The patient had difficulty swallowing and required nasogastrostomy feeding for failure to thrive. He had chronic diarrhea, recurrent bronchopulmonary infections and complicated bronchomalacia occurred. A brain MRI showed mild delay of myelination, mild dilatation of the lateral ventricles and hypoplasia of the splenium of the corpus callosum (Fig. 1B,C).

MOLECULAR AND CYTOGENETIC ANALYSIS

G-banded chromosomal analysis showed interstitial deletion of 16q21–22.1 (Fig. 2A). It was revealed as de novo, because both his parents showed normal karyotyping. Deletion of 22q11.2 region was not found by conventional FISH analysis with TUPLE as a probe (Vysis, IL, USA) (data not shown). Detailed FISH analysis using Human BAC clones was carried out to determine the precise lesion of the deletion according to a method described elsewhere (Fig. 2B) [Shimokawa et al., 2004]. The results of the FISH analysis are summarized in Table I. The chromosomal locations of the Human BAC clones are from UCSC Genome Browser (http://genome.ucsc.edu/).

Haplotype analysis of this family was performed using the microsatellite marker, D16S3129, on 16q21. Primer information was also retrieved from the UCSC Genome Browser (http://genome.ucsc.edu/). Genomic DNAs were obtained from family members, and subsequent PCR amplification was performed according to the standard method. The amplicons were separated by acrylamide gel electrophoresis and visualized by ethidium bromide staining. As the patient had bands in common only with his father, it was deduced that the maternally derived allele at this locus was deleted in the patient.

TABLE I. Summary of FISH Analyses

Clone name[a]	Location: Chromosome band	Location: Nucleotide position[b] Start	Location: Nucleotide position[b] End	Result of FISH
RP11-19E17	16q21	58,616,483	58,770,461	Normal
RP11-89G14	16q21	59,355,949	59,380,997	Normal
RP11-110A13	16q21	59,894,560	60,054,882	Normal
RP11-828P4	16q21	60,811,619	60,961,494	Deletion
RP11-25K3	16q21	62,180,775	62,331,661	Deletion
RP11-89C10	16q22.1	64,421,381	64,579,967	Deletion
RP11-5A19	16q22.1	65,622,600	65,775,715	Deletion
RP11-462K4	16q22.1	67,158,261	67,228,107	Deletion
RP11-123C5	16q22.1	67,553,305	67,731,523	Deletion
RP11-14J15	16q22.1	68,192,246	68,289,674	Normal
RP11-343L1	16q22.1	69,210,076	69,365,098	Normal
RP11-113E3	16q22.2	69,787,194	69,942,894	Normal
RP11-58M3	16q22.2	70,170,265	70,355,260	Normal
RP11-90L19	16q22.2	70,848,592	71,029,614	Normal
RP11-7J20	16q22.3	71,909,257	72,091,524	Normal

[a]BAC clones located to 16q21–22.1 used as probes in FISH study.
[b]Chromosomal location or nucleotide position are from the UCSC database (May 2004).

DISCUSSION

TOF associated with PA and MAPCA, identified in this patient, is a complex and extremely heterogeneous CHD that has not been accurately defined. MAPCA are likely to be dilated bronchial arteries, and appear to have a limited growth potential. The term

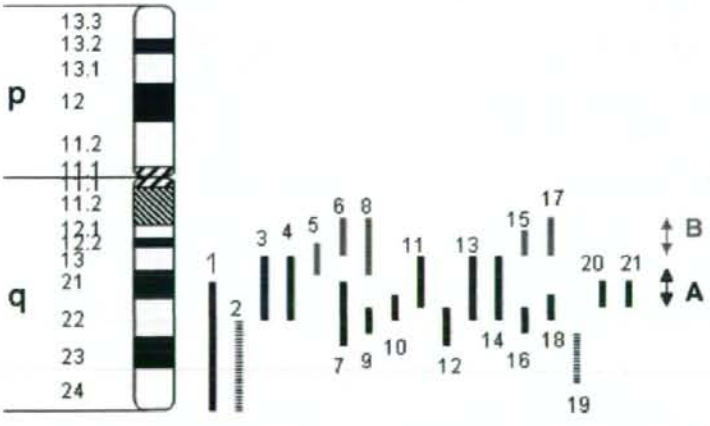

FIG. 3. Schematic representation of the chromosomal region. Bars with arrows indicate the regions of two common deletions A (black) and B (gray). The deleted regions of the reported patients are depicted by vertical lines. Black and gray lines indicate that the patients share different common regions, respectively. Broken lines indicate the patients whose deletion region are not common to either A and B. The numbers indicate references as following: (1) Fryns et al. [1977]; (2) Taysi et al. [1978]; (3) Fryns et al. [1981]; (4) Lin et al. [1983]; (5) Elder et al. [1984]; (6) Hoo et al. [1985]; (7) Rivera et al. [1985]; (8) Krauss et al. [1987]; (9) Natt et al. [1987, 1989] [TH]; (10) Cooke et al. [1987]/Callen et al. [1993] [ID5]; (11) Naritomi et al. [1988]; (12) Natt et al. [1989] [KS]; (13) Casamassima et al. [1990]; (14) Edelhoff et al. [1991]; (15) Schuffenhauer et al. [1992]; (16) Fujiwara et al. [1992]; (17) Doco-Fenzy et al. [1994]; (18) Callen et al. [1993] [ID4]; (19) Callen et al. [1993] [ID7]; (20) Khan et al. [2006]; (21) Present case.

TABLE II. Summary of Clinical Features of Reported Patients Who Share Common Region 16q21–q22

	(1) Fryns et al. [1977]	(3) Fryns et al. [1981]	(4) Lin et al. [1983]	(7) Rivera et al. [1985]	(9) Natt et al. [1987, 1989]	(10) Cooke et al. [1987]/ Callen et al. [1993] [ID5]	(11) Naritomi et al. [1988]	(12) Natt et al. [1989] [KS]	(13) Casamassima et al. [1990]	(14) Edelhoff et al. [1991]	(16) Fujiwara et al. [1992]	(18) Callen et al. [1993] [ID4]	(20) Khan et al. [2006]	(21) Present case
Growth														
Small for dates	+	+	–	+	NS	+	+	NS	–	–	–	–	+	+
Postnatal growth <3rd centile	+	+	+	+	NS	+	+	+	–	–	+	+	–	+
Microcephaly	+	+	+	+	NS	+	+	NS	–	–	+	+/–	+	+
Failure to thrive	+	+	+	NS	NS	+	+	NS	+	+	+	+	–	+
CNS and development														
Psychomotor retardation	NS	+	+	NS	+	+	+	+	+	+	+	+	+	+
Hypotonia	+	+	+	–	NS	+	+	+	+	+	+	+	NS	+
Feeble suck	+	+	+	+	NS	+	NS	NS	+	NS	+	+	+	+
Hydrocephalus/enlarged ventricles	–	+	–	NS	NS	–	+	NS	NS	NS	–	–	–	+
Craniofacial														
Large anterior fontanelle	+	+	+	+	NS	+	+	NS	NS	+	+		+	+
High forehead	+	+	+	+	+	+	+	+	+	–	+	+/–	+	+
Diastasis cranial sutures	+	+	+	+	NS	+	+	NS	NS	+	+	+	+	–
Prominent metopic sutures	+	+	+	+	NS	+	NS	NS	+	–	+	–	–	–
Broad flat nasal bridge	+	–	+	+	–	+	+	+	–	+	+	+	+	+
Hypertelorism	+	–	+	+	–	–	+	–	+	+	+	–	+	+
Low set dysmorphic ears	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Small palpebral fissures	+	+	+	+	NS	–	–	–	+	–	–	–	+	+
Upward slanting palpebral fissures	+	–	+	–	+	+	–	+	–	–	+	–	+	+
Micrognathia	+	+	+	+	–	+	–	–	+	+	–	+	+	+
High arched palate	–	–	–	+	C	+	NS	NS	+	+	+	+	C	C
Short neck	+	+	+	+	–	+	+	–	+	+	–	NS	+	+
Thorax and abdomen														
Congenital heart defect	+ VSD	–	+ ECD	+ AC	NS	+ TAVPR	–	+ AS	–	+ CAVC	–	+ PA	+ VSD	+ TOF
Narrow thorax	+	+	+	+	NS	+	–	NS	–	+	+	–	–	–
Ectopic anus (gastrointestinal anomalies)	+	+	+	NS	NS	+	–	NS	–	–	–	–	–	–
Renal cystic dysplasia/ hypoplasia	–	+	–	NS	+	+	NS	NS	–	–	–	–	NS	–
Extremities														
Flexed fingers	+	–	+	+	NS	+	NS	+	–	+	NS	+	NS	–
Small hands and feet	–	+	+	NS	NS	–	NS	NS	NS	NS	–	–	+	–
Bilateral simian creases	+	–	+	NS	NS	–	+	NS	NS	+	+	–	R	+
Malposition of toes	+	–	+	NS	NS	+	NS	+	+	+	NS	–	NS	+
Talipes eqinovarus/ calcaneovaigus (foot deformity)	+	–	+	+	+	+	+	NS	+	–	NS	–	NS	–
Broad first toe	+	+	+	NS	NS	+	+	NS	+	+	–	–	NS	–

NS, not stated; C, cleft palate; R, right side only; VSD, ventricular septal defect; ECD, endocardial cushion defect; AC, aortic coarctation; TAPVR, total anomalous pulmonary venous return; AS, aortic stenosis; CAVC, common atrio ventricular canal; PA, pulmonary atresia; TOF, tetralogy of Fallot.

used to describe this condition is also controversial and some researchers are using "PA, ventricular defect and MAPCA" to describe it [Tchervenkov and Roy, 2000]. TOF associated with PA and MAPCA has a familial association with 22q11.2 deletion syndrome. Vesel et al. [2006] reported prenatally diagnosed patients and underlying genetic causes, and the prevalence of 22q11.2 deletion was 24% (6/25) in their series. There are some similar reports showing the prevalence of 22q11.2 deletion as 23–40% [Chessa et al., 1998; Hofbeck et al., 1999; Anaclerio et al., 2001; Mahle et al., 2003]. The clinical courses of four patients reported by Yamagishi et al. [2002] are similar to that of our patient, since bronchomalacia associated with CHD was found, and each patient was diagnosed as having 22q11.2 deletion syndrome. However, in our patient, deletion of 22q11.2 region was not present.

Although two critical regions for the main clinical findings of interstitial deletion syndrome of 16q have been discussed in the literature, 14 patients (including this patient) among the 21 reported patients with 16q deletion syndrome showed the deletion around the 16q21–q22 region, which is the most common region (ranges of the deletions shown in Fig. 3) [Fryns et al., 1977, 1981; Taysi et al., 1978; Lin et al., 1983; Rivera et al., 1985; Cooke et al., 1987; Natt et al., 1987, 1989; Naritomi et al., 1988; Casamassima et al., 1990; Edelhoff et al., 1991; Fujiwara et al., 1992; Callen et al., 1993; Chen et al., 1998]. Among these 14 patients with deletions of the 16q22 region including our patient, nine patients showed CHD (64%), which is the most common major organ malformation [Goldmuntz, 2004] (Table II). Recent development of the technology identified many genetic etiologies of CHD. *GATA4* is one of the most well-known genes related to structural CHD, especially ASD [Garg et al., 2003]. *TBX1* is also known as a disease-causing gene for conotruncal cardiac defects [Yagi et al., 2003]. However, the genes which we know are only small parts of CHD. Examinations of patients with syndromes of known chromosomal abnormalities provided insight into the related forms of dysmorphism. Thus, detailed examinations of the aberrant chromosome can help identification of the related genes, and there might be a responsible gene for CHD in the region of 16q22.1.

However, there are two ambiguities. The first is that the types of CHD reported in 16q deletion syndrome are variable and there is no common feature, i.e. ventricular septum defect [Fryns et al., 1977], endocardial cushion defect [Lin et al., 1983], aortic coarctation [Rivera et al., 1985], total anomalous pulmonary venous drainage [Cooke et al., 1987], aortic stenosis [Natt et al., 1989], common atrioventricular canal and patent ductus arteriosus [Edelhoff et al., 1991], pulmonary atresia with hypoplastic right ventricle and tricuspid valve [Callen et al. 1993], and ventricular septal defect and pulmonary artery branch stenosis [Khan et al., 2006]. TOF associated with PA and MAPCA has been reported only in our patient. The second ambiguity is that the smallest region for CHD has not been confirmed, since many previous studies did not include detailed investigations of the deleted regions by advanced molecular and/or cytogenetic analyses. Accordingly, we should accumulate more information to identify the responsible genes for CHD which might be located in this area.

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Research Letter

No Mutation in RAS-MAPK Pathway Genes in 30 Patients With Kabuki Syndrome

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To the Editor:

Kabuki syndrome (KS, OMIM 147920) also known as Niikawa–Kuroki syndrome, is a multiple congenital anomaly/mental retardation (MCA/MR) syndrome characterized by a distinctive facial appearance resembling the Kabuki actor's make-up, skeletal abnormalities, joint hypermobility, dermatoglyphic abnormalities, postnatal growth retardation, occasional visceral anomalies and immune abnormalities. The cause of KS remains unknown, even though a large number of patients from a variety of ethnic groups have been reported since 1981 [Wessels et al., 2002]. The prevalence was estimated to be 1/32,000 in Japan [Niikawa et al., 1988] and 1/86,000 in Australia and New Zealand [White et al., 2004]. Although most cases were sporadic, at least 14 familial cases have been reported. The equal male-to-female ratio of patients, and parent-child transmissions in some familial cases suggest an autosomal dominant of inheritance [Niikawa et al., 1988; Matsumoto and Niikawa, 2003]. At least six autosomal structural abnormalities have been reported in patients with KS or KS-like features [Matsumoto and Niikawa, 2003], but no concordant specific cytogenetic lesion have been found.

It is less likely that a large-scale genomic rearrangement is the common cause of KS, because array-based comparative genome hybridization (array-CGH) did not detect any abnormality in previously reported 8p22–p23.1, and in whole genome with 1.2/1.5 megabase resolution [Hoffman et al., 2005; Schoumans et al., 2005; Miyake et al., 2006]. Although it was reported that a patient with KS had a de novo 250 kilobase microdeletion of the exon 5 region in *C20orf133* gene, 19 additional patients with KS did not have any mutations or copy number changes of the gene [Maas et al., 2007].

Recently, germline mutations in some genes involving the RAS-mitogen-activated protein kinase (RAS-MAPK) signal transduction pathway have been shown to be causes of multiple congenital anomaly

This article contains supplementary material, which may be viewed at the American Journal of Medical Genetics website at http://www.interscience.wiley.com/jpages/1552-4825/suppmat/index.html.

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syndromes; i.e. Noonan syndrome due to *PTPN11* [Tartaglia et al., 2001], *KRAS* [Schubbert et al., 2006], *SOS1* [Roberts et al., 2007; Tartaglia et al., 2007] and *RAF1* [Pandit et al., 2007; Razzaque et al., 2007]; Costello syndrome due to *HRAS* [Aoki et al., 2005]; cardio-fadio-cutaneous (CFC) syndrome due to *KRAS* and *BRAF* [Niihori et al., 2006], and *BRAF*, *MEK1* and *MEK2* [Rodriguez-Viciana et al., 2006]. These achievements encouraged us that a disturbance of certain transcriptional factors or oncogenes related to the pathway may cause KS as an MCA syndrome due to their variety functions. To test the hypothesis, we screened in 30 patients with KS (14 females and 16 males) for mutations in 16 genes involving the RAS-MAPK pathway.

Experimental procedures were approved by the Committee for the Ethical Issues on Human Genome and Gene Analysis in Nagasaki University. Genomic DNA was extracted from their peripheral blood leukocytes or from EBV-transformed lymphoblastoid cells established after obtaining informed consent from all subjects and/or their parents. The selected genes for mutation analysis were following: *PTPN11*, *SOS1*, *GRB2*, *HRAS*, *KRAS*, *ERAS*, *NRAS*, *ARAF*, *BRAF*, *RAF1* (*CRAF*), *MEK1*, *MEK2*, *RASA1*, *RASA2*, *RASA3* and *RASA4*. These accession numbers are respectively NM_002834, NM_005633, NM_203506, NM_005343 and NM_176795 (HRAS1 and H-RAS), NM_033360 and NM_004985 (KRAS isoform a and b), NM_181532, NM_002524, NM_001654, NM_004333, NM_002880, NM_002755, NM_030662, NM_002890 and NM_022650 (RASA1 isoform 1 and 2), NM_006506, NM_007368, and NM_006989. Genomic sequences were retrieved from the UCSC genome browser (assembly: March 2006; http://genome.ucsc.edu/). The entire coding exons and splice junctions of the genes were directly sequenced using BigDye sequencing kit ver.3.1 (Applied Biosystems, Foster City, CA) and an automated sequencer Model 3100 (Applied Biosystems). PCR conditions and primer sequences are available in the online supplement (see the online supplementary file at http://www.interscience.wiley.com/jpages/1552-4825/suppmat/index.html).

In 227 coding exons of the 16 genes analyzed among 30 patients with KS, we found 27 base substitutions (Table I). Nine base substitutions lead to nonsynonymous amino acid changes. Two missense mutations in *RASA1* gene in two patients with KS were detected, which were not found in 86 phenotypically normal Japanese controls, but each mutation was detected in only one patient. Unfortunately DNA samples from their parents were not available. TaqMan real-time quantitative PCR assay for the *RASA1* gene in 30 patients did not show any copy number changes (data not shown). Mutations in *RASA1*, most of them results in premature termination codon, are known as a cause

TABLE I. Nucleotide Changes Found in Genes Analyzed in the RAS-MAPK Pathway in 30 Patients With Kabuki Syndrome

		Change of				
	Gene	Nucleotide	Amino acid	Number of patient(s)	SNP ID	AF
Non-synonymous	*RASA1*	c.73G>A	A25T	1	NR	0.000
		c.473C>G	S158C	1	NR	0.000
	RASA4	c.379T>C	W127R	7	NR	0.800
		c.381G>C	W127R	9	NR	0.900
		c.401G>A	R134Q	12	NR	0.806
		c.674T>C	V225A	6	NR	0.051
		c.728G>A	R243Q	7	NR	0.063
		c.1054A>G	M352V	8	rs746316	
		c.1103T>C	L368P	4	rs886343	
Synonymous	*SOS1*	c.195A>C	R65R	1	NR	0.045
		c.1230G>A	Q410Q	1	NR	0.000
	BRAF	c.1689C>G	G563G	1	NR	0.000
	RASA4	c.330C>T	V110V	22	NR	0.847
		c.336C>T	P112P	25	NR	0.847
	KRAS	c.519T>C	D173D	10	rs1137282	
	HRAS	c.81T>C	H27H	9	rs2227994	
	BRAF	c.1929A>G	G643G	4	rs1042179	
	RAF1	c.1629A>G	T543T	1	rs5746244	
	MEK2	c.453C>T	D151D	5	rs17851657	
		c.660C>A	I220I	20	rs11539507	
	RASA1	c.3067T>C	L1023L	3	rs3747704	
	RASA2	c.2028T>C	N672N	16	rs295322	
		c.2172G>A	L720L	18	rs295323	
	RASA3	c.1326T>C	T442T	12	rs2274717	
	RASA4	c.339T>C	D113D	4	rs11547191	
		c.1512C>T	A504A	3	rs739735	
		c.2253C>T	G751G	7	rs3099742	

AF, allele frequency among 82–89 phenotypically normal Japanese controls; NR, not registered in NCBI database.

of capillary malformation-arteriovenous malformation (CM-AVM) [Boon et al., 2005], but the manifestations of CM-AVM are so different from that of KS that it is less likely responsible for KS. Seven base substitutions of the nonsynonymous amino acid changes were confirmed as single nucleotide polymorphisms (SNPs) listed in the database of SNP or found in 82–89 normal Japanese controls. Synonymous changes were found as 18 base substitutions including 13 SNPs registered, 3 base changes found in the controls, and 2 base changes not found in the controls. Consequently, no pathogenic mutations were detected in any of the genes analyzed in RAS-MAPK pathway and in any of the patients with KS examined. Although our results do not totally rule out the role of RAS-MAPK pathway in KS, it is less likely that the genes in this pathway are associated with KS.

Since there has been no clue to identify the putative gene causative for KS, candidate gene approaches would be valuable in a view of "inborn errors of development". In this connection, transforming growth factor β receptors (*TGFBR*) 1 and *TGFBR2*, relating *IRF6* gene which is causative for van der Woude syndrome (VWS), was added to candidate genes because of specific lower lip pits with VWS and with KS in common, but the two genes did not show any mutations and copy number changes among 14 patients with KS [Bottani et al., 2006]. We may need to perform an intensive PCR-based mutation screening in the genes involving the TGF-β intracellular signaling pathways.

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ORIGINAL ARTICLE

Microarray comparative genomic hybridization analysis of 59 patients with schizophrenia

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Abstract Schizophrenia is a common psychiatric disorder with a strong genetic contribution. Disease-associated chromosomal abnormalities in this condition may provide important clues, such as *DISC1*. In this study, 59 schizophrenia patients were analyzed by microarray comparative genomic hybridization (CGH) using custom bacterial artificial chromosome (BAC) microarray (4,219 BACs with 0.7-Mb resolution). Chromosomal abnormalities were found in six patients (10%): 46,XY,der(13)t(12;13)(p12.1;p11).ish del(5)(p11p12); 46,XY, ish del(17)(p12p12); 46,XX.ish dup(11)(p13p13); and 46,X,idic(Y)(q11.2); and in two cases, mos 45,X/46XX. Autosomal abnormalities in three cases are likely to be pathogenic, and sex chromosome abnormalities in three follow previous findings. It is noteworthy that 10% of patients with schizophrenia have (sub)microscopic chromosomal abnormalities, indicating that genome-wide copy number survey should be considered in genetic studies of schizophrenia.

Keywords Schizophrenia · Chromosomal abnormality · Array comparative genomic hybridization · Copy number variation

Introduction

Schizophrenia is a common psychiatric disorder involving approximately 1% of the population worldwide. Family, twin, and adoption studies suggest genetic factors contribute to this illness (Lang et al. 2007; McGuffin et al. 1995). Meta-analysis including 18 genome scans revealed strong evidence at chromosomal regions 22q, 8p, and 13q

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as the susceptibility loci (Badner and Gershon 2002), and another meta-analysis of 20 genome-wide scans suggested regions of chromosomes 2q, 5q, 3p, 11q, 6p, 1q, 22q, 8p, 20q, and 14p as the significant loci (Lewis et al. 2003). Chromosomal abnormalities in patients with schizophrenia may provide useful information regarding the susceptible loci (Bassett et al. 2000). Disrupted in schizophrenia 1 (*DISC1*) gene isolated from a large Scottish family with t(1;11)(q42.1;q14.3) and high risk of schizophrenia in velo-cardio-facial syndrome (VCFS) with a 22q11 deletion are good examples (Arinami 2006; Millar et al. 2000; Murphy 2002). Some linkage and association studies support that schizophrenia could be associated with *DISC1* and genes at 22q11 (Chubb et al. 2008; Liu et al. 2002; O'Donovan et al. 2003; Shifman et al. 2002).

Microarray technologies have now become practical tools for detection of submicroscopic copy number changes. Using custom bacterial artificial chromosome (BAC) microarray (4,219 BACs at 0.7-Mb resolution), we analyzed 59 patients with schizophrenia. Chromosomal abnormalities found in this study are presented.

Materials and methods

Subjects

A total of 59 subjects (31 men and 28 women) with schizophrenia were recruited in this study. Forty-one had family history. Diagnosis was made for each patient according to the *Diagnostic and Statistical Manual of Mental Disorders*, 4th edition (DSM-IV) criteria on the basis of unstructured interviews and information from medical records. Participants were excluded if they had organic brain diseases, including head injury and infection, or if they met criteria for alcohol/drug dependence. After written informed consent, genomic deoxyribonucleic acid (DNA) from lymphoblastoid cell line (LCL) of all patients was isolated using DNA isolation systems [Quick Gene-800 (Fujifilm, Tokyo, Japan) and/or NA-3000 (Kurabo, Osaka, Japan)]. Micorarray comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH) analysis were performed using materials from LCL. Peripheral blood lymphocytes were reevaluated in ID394, MZ102, and MZ127, but could not be obtained for reexamination in ID67, ID345, or ID391. Only parents of ID345 subjects were available for familial analysis. Other parents or sibs could not be evaluated. Experimental protocols were approved by the Committee for Ethical Issues at Yokohama City University School of Medicine.

Microarray CGH analysis

Comparative genomic hybridization analysis was performed using our custom BAC microarray containing 4,219 BAC clones, as previously described (Saitsu et al. 2008). In brief, after complete digestion using *Dpn*II, subject's DNA was labeled with Cy-5 dCTP (Amersham Biosciences, Piscataway, NJ), and reference DNA was labeled with Cy-3 deoxycytidine triphosphate (dCTP) (Amersham Biosciences) using the DNA random primer Kit (Invitrogen). Prehybridization, probe hybridization, washing, and drying steps for arrays were preformed on a Tecan hybridization station HS400 (Tecan Japan, Kawasaki, Japan). Arrays were scanned by GenePix 4000B (Axon Instruments, Union City, CA, USA) and analyzed using GenePix Pro 6.0 (Axon Instruments). The signal intensity ratio between patient and control DNA was calculated from the data of the single-slide experiment using the ratio of means formula (F635 mean − B635 median/F532 mean − B532 median) according to GenePix Pro. 6.0. The standard deviation was calculated from the data of all clones. We regarded the signal ratio as abnormal if it ranged out of ±3 standard deviations (SD). Clones showing abnormal copy number were checked to see whether they were in the position of previously registered copy number variations using the Human Genome Variation Database (http://www.hgvbase.org/) (Iafrate et al. 2004). Unregistered changes were considered for further confirmation. Genome position was based on the UCSC genome browser Human Mar. 2006 (hg18) assembly.

Fluorescence in situ hybridization

To confirm status of clones with a possibly abnormal copy number, FISH was performed, as previously described (Shimokawa et al. 2005). BAC DNA was labeled with SpectrumGreen™-11-deoxyuridine triphosphate (dUTP) or SpectrumOrange™-11-dUTP (Vysis, Downers Grove, IL, USA) by nick translation and denatured at 70°C for 10 min. Probe-hybridization mixtures (15 μl) were applied on chromosomes, incubated at 37°C for 16–72 h, then washed and mounted in antifade solution (Vector, Burlingame, CA, USA) containing 4'-6'-diamidino-2-phenylindole (DAPI). Photographs were taken on an AxioCam MR CCD fitted to Axioplan2 fluorescence microscope (Carl Zeiss, Oberkochen, Germany). In ID394 and MZ102, we counted 100 interphase nuclei to validate the number of cells with X aneuploidy, as well as 30 metaphases.

Results and discussion

Six patients showed chromosomal abnormalities (10%, 6/59) (Table 1). As we could not obtain materials from most

Table 1 Summary of six patients with (sub)microscopic chromosomal rearrangements

Patient	Gender	FH	Karyotype	Size of imbalance
ID67	M	No	46,XY,der(13)t(12;13)(p12.1; p11).ish del(5)(p11p12)	1.7 Mb deletion (chr.5) 23.1 Mb gain (chr.12)
MZ127	F	Yes	46,XX.ish dup(11)(p13p13)	430 bp (?) gain (chr.11)
ID345	M	No	46,XY, ish del(17)(p12p12)	1.3 Mb deletion (chr.17)
MZ102	F	Yes	Mos45,X/46,XX	Whole X loss (mosaic)
ID394	F	Yes	Mos45,X/46,XX	Whole X loss (mosaic)
ID391	M	Yes	46,X,idic(Y)(q11.2)	Yq12-qter deletion Yq11.23-Yq12 gain

FH family history of schizophrenia and/or other psychiatric disorders

of their parents and sibs, heritability of the abnormalities could not fully be investigated. According to our experiences of microarray CGH analysis of more than 200 Japanese patients associated with mental-retardation-related disorders, all chromosomal abnormalities described here were never detected. Thus, it is less likely that the changes are polymorphisms.

In ID67, arr cgh 5p12p12(RP11-1037A10 → RP11-929P16) × 1, 12pterp12.1(GS-124K20 → RP11-12D15) × 3 was found. A 23.2-Mb copy number gain from 12pter to 12p12.1 (chr12: 0–23,176,547 bp) was detected (Fig. 1a). G-banded chromosomal analysis revealed that 12pter-12p12.1 was translocated to 13p11 (Fig. 1a). The 12p12.1 translocation breakpoint was localized between two BAC clones, RP11-35A22 and RP11-349E13, by FISH (chr12: 23,176,547–23,861,227 bp) (data not shown). Additionally, a 1.7-Mb submicroscopic deletion at 5p12 from RP11-1037A10 to centromeric sequence gap (chr5: 44,778 009–46,437 323 bp) was also found in this patient (Fig. 1a). The 12p trisomy is recognized as multiple congenital anomalies/mental retardation (MCA/MR) syndrome characterized by dysmorphic face, heavy birth weight, foot deformities, hypotonia, and mental retardation (Allen et al. 1996). A previous study suggested that partial duplication of 12pter-p13.2 is sufficient for recognizable phenotype of 12p trisomy (Rauch et al. 1996). The 23.1-Mb duplicated region contained at least 229 genes. Dysmorphic facial features of 12p trisomy (Rauch et al. 1996) were not recognized in this patient. It is interesting that ID67 also had a 1.7-Mb deletion at 5p12, containing two genes, *MRPS30* (the mitochondrial ribosomal protein S30 gene) and *HCN1* (the hyperpolarization-activated cyclic nucleotide-gated potassium channel 1 gene). It is worth noting linkage findings within the vicinity of this region in Costa Rican schizophrenia samples (Cooper-Casey et al. 2005). *HCN1* is an intriguing candidate gene. The general *Hcn*1 loss in mice led to a defect in the learning of motor tasks, and specific deletion of the gene in forebrain neurons resulted in an unexpected enhancement of spatial learning and memory (Herrmann et al. 2007; Nolan et al. 2003). ID67 (a 72-year-old male) developed psychotic symptoms (delusions, hallucinations, and psychomotor excitement) at age 20 years. He had received electroconvulsive therapy many times and continuous sleep therapy until antipsychotic medication (chlorpromazine) was introduced at age 23 years. Since the onset of the illness, he has spent most of his life in psychiatric hospitals because of exacerbations of psychotic episodes and marked deterioration of social functions. Intelligent quotient (IQ) at 72 years was 72. He had no family history of major psychosis within the first-degree relatives.

In MZ127, arr cgh 11p13p13(RP11-51J14) × 3 was recognized. Duplication of RP11-51J14 at 11p13 (chr11: 33,302,231–33,302,660 bp) was confirmed by FISH using LCL and peripheral blood lymphocytes (Fig. 1b). According to the genome browser, the size of RP11-51J14 is 430 bp, indicating that the reference sequence is somehow odd and may contain a deletion overlapping with RP11-51J14 as FISH signals of RP11-51J14 are strong enough to detect on a microscope, suggesting that its size is at least >10 kb. *HIPK3* (the homeodomain interactive protein kinase 3 gene) was corresponding to this clone. HIPK3 is a Fas-associated death-domain (FADD)-interacting kinase involved in apotosis (Curtin and Cotter 2003), remaining unknown in relation to schizophrenia. MZ127 (42-year-old woman) presented with epilepsy at age 12 years and has had recurrent depression and slight mania since age 29 years. She began to exhibit auditory hallucination, not synchronizing with mood swing, and was diagnosed as schizophrenia at 40 years. Her mother and sister suffered from major depression and schizophrenia, respectively. Her father committed suicide induced by depression.

In ID345, arr cgh 17p12p12(RP11-78J16 → RP11-103P10) × 1 was found, as previously described (Ozeki et al. 2008). The deletion from RP11-246F16 to RP11-103P10 (chr17: 14,061,460–15,374,745 bp) is 1.4 Mb, compatible with the common deletion found in approximately 85% of hereditary neuropathy with liability to pressure palsies (HNPP; OMIM #162500) (Stogbauer et al.

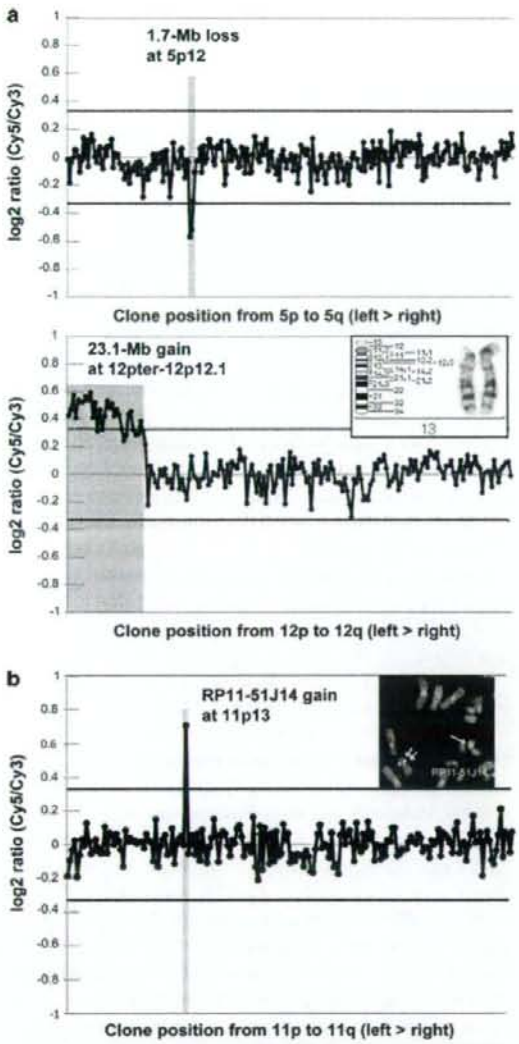

Fig. 1 Results of microarray comparative genomic hybridization (*CGH*) in ID67 (**a**) and MZ127 (**b**). Chromosomes 5 (*upper*) and 12 (*lower*) are displayed (**a**). The karyotype is arr cgh 5p12p12(RP11-1037A10 → RP11-929P16) × 1, 12pterp12.1(GS-124K20 → RP11-12D15) × 3. Partial karyotype clearly shows a 12pter-p12.1 segment is translocated to 13p11. Chromosome 11 is presented (**b**) . The karyotype is arr cgh 11p13p13(RP11-51J14) × 3. RP11-51J14 at 11p13 is duplicated

2000). The deletion was also identified in his father's chromosomes from peripheral blood lymphocytes. He suffered from auditory hallucination and delusion of persecution and received antipsychotic treatment at age 19. Neurological examination did not reveal any manifestations of HNPP (Ozeki et al. 2008). Pareyson et al. (1996) reported that about 25% of individuals with HNPP deletion are asymptomatic. The peripheral myelin protein 22 gene (*PMP22*) may be a candidate that is not only expressed in the peripheral nervous system but also in the central nervous system (Ohsawa et al. 2006), this being supported by linkage studies of psychotic bipolar disorder (Park et al. 2004) and schizophrenia (Owen et al. 2004). No family history regarding psychiatric disorders was observed in ID345.

Entire X chromosome copy number aberration was suspected in two patients, ID394 and MZ102 (data not shown). FISH analysis using RP11-65B15 at Xq23 revealed mosaic monosomy of chromosome X: mos45,X[41]/46,XX[59] in ID394 and mos45,X[84]/46,XX[16] in MZ102. X aneuploidy is well known to be seen in elderly normal females (Stone and Sandberg 1995). ID394 and MZ102 were 67 and 38 years old, respectively. The fraction of cells with X monosomy was very high (84% and 41%) in lymphoblastoid cell lines of these patients. Reevaluation of peripheral blood lymphocytes showed mos 45,X[7]/46,XX[98] in ID394 and mos 45,X[4]/46,XX[96] in MZ102. These findings may support involvement of X-chromosomal abnormalities in schizophrenia (Kumra et al. 1998; Kunugi et al. 1999), but mosaic X monosomy is also found in age-matched normal controls (Toyota et al. 2001). ID394 (a 67-year-old woman) developed psychotic symptoms (paranoid delusion and hallucinations) at age 31 years when she delivered her second child. Since then, she had been admitted to a psychiatric hospital three times (each for a few months). She quit her job as a pharmacist after the onset of the illness and has lived as a housewife. She has been managed by antipsychotic medications without major exacerbation for the past decade. The second child developed schizophrenia-like symptoms, including social withdrawal and lack of volition. MZ102 (a 38-year-old woman) exhibited psychomotor excitement and was diagnosed as having schizophrenia at age 23 years. Her father showed psychotic disorder, and her uncle had schizophrenia. In ID391, arr cgh Ypterq11.23(GS-98C4 → RP11-214M24) × 3, Yq11.23qter(RP11-263C17 → RP11-80F8) × 1 was identified. FISH analysis using BACs, RP11-74L17 at PAR1, RP11-375P13 at Yp11.2, RP11-655E20 at Yq11.2, and RP11-80F8 at Yq12 revealed the isodicentric Y chromosome [46,X,idic(Y)(q11.2)] (data not shown). Previously, two cases of idic(Yp) were reported in schizophrenia, although idic(Yp) is one of the most common rearrangements in the Y chromosome (Nanko et al. 1993; Yoshitsugu et al. 2003). ID391 (a 29-year-old man) developed hallucinations and abnormal sense of self at age 21 years, when he was admitted to a psychiatric hospital for 3 months. Since then, his illness has been well controlled by antipsychotic medication. He quit university after the onset

of illness and has not obtained a job, suggesting deterioration of functioning. His younger sister (apparently without the Y chromosome) has schizophrenia. Thus, contribution of sex chromosomal abnormalities found in this study is less likely.

Four microarray CGH studies of schizophrenia were reported: 1,440 BAC microarray for 30 patients, 2,460 BAC microarray for 35 patients, a tiling-path microarray consisting of ~36,000 BACs for 93 patients, and high-resolution microarrays (85,000–2,100,000 oligos) for 150 patients (Kirov et al. 2008; Moon et al. 2006; Walsh et al. 2008; Wilson et al. 2006). We could not replicate any similar abnormalities, though microarray platforms were all different in terms of clones and genome coverage. In this study, (sub)microscopic rearrangements were detected in 10% of patients. Similarly, 15% of patients analyzed by high-resolution microarrays were found to possess submicroscopic chromosomal changes (Walsh et al. 2008). Various kinds of recurrent and unique submicroscopic changes were found in 10–17% of idiopathic mental retardation and 7% of autism by microarray CGH analysis (Miyake et al. 2006; Sebat et al. 2007; Zahir and Friedman 2007). Importantly, a 22q13 deletion (in autism) involving Sh3 and multiple ankyrin repeat domains 3 (*SHANK3*), whose point mutation was related to autism (Durand et al. 2007), strongly supports this approach as one of the most powerful and straightforward strategies in neuropsychiatric disorders.

In conclusion, microarray technologies could provide good opportunity to identify chromosomal copy number changes in relation to mental and psychiatric disorders, and genome-wide copy number survey should be considered in genetic studies of these disorders.

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Clinical Report

Paternal Somatic Mosaicism of a *TGFBR2* Mutation Transmitting to an Affected Son With Loeys–Dietz Syndrome

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We report on somatic mosaicism of a *TGFBR2* missense mutation, c.1336G > A (D446N). The affected son with the heterozygous mutation was previously reported [Sakai et al. (2006); Am J Med Genet A 140A:1719–1725]. Further evaluation indicates his clinical condition is Loeys–Dietz syndrome. Parental blood samples were studied to confirm whether the propositus' mutation was a de novo change, and suggested a trace of the mutation in the father. DNAs extracted from blood leukocytes, buccal cells, hair root cells, and nails in the father indicated 52%, 25%, 0%, and 35% of cells harbored the mutation, respectively. This is the first detailed report of somatic mosaicism of a *TGFBR2* mutation. © 2008 Wiley-Liss, Inc.

Key words: mosaicism; *TGFBR2*; mutation; Marfan syndrome; Loeys–Dietz syndrome

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INTRODUCTION

Marfan syndrome (MFS, OMIM #154700) is an autosomal dominant connective tissue disorder characterized by involvement of skeletal, ocular, and cardiovascular systems. *FBN1* abnormalities are the major cause of MFS, but *TGFBR2* aberrations were also found in a subset of MFS [Dietz et al., 1991; Mizuguchi et al., 2004; Boileau et al., 2005; Mizuguchi and Matsumoto, 2007]. Furthermore *TGFBR2* and *TGFBR1* mutations were reported in Loeys–Dietz syndrome (LDS, OMIM #610168), a new MFS-related disorder [Loeys et al., 2005]. Abnormalities of TGF-β signaling are now highlighted as an important aspect in pathogenesis of MFS and MFS-related conditions [Boileau et al., 2005; Mizuguchi and Matsumoto, 2007].

Wide variability of phenotypes in connective tissue disorders is well known. As for MFS, this may be attributable partly to somatic mosaicism of *FBN1* mutations. However somatic mosaicism was confirmed in only three families [Montgomery et al., 1998; Collod-Beroud et al., 1999; Tekin et al., 2007] and germline mosaicism was inferred in one family [Rantamaki et al., 1999] among more than 600 *FBN1* mutations registered in the UMD-FBN1 database (http://www.umd.be:2030/) [Collod-Beroud et al., 2003]. Somatic mosaicism could be very rare or could be hardly detectable with regular methods in *FBN1* analysis. A similar scenario could be expected in LDS, and somatic mosaicism of a *TGFBR2* mutation (R537G) in a father of the LDS patient was briefly mentioned [Loeys et al., 2006].

We encountered a family with a propositus with a *TGFBR2* mutation, c.1336G > A (D446N), whose father showed somatic mosaicism for the mutation. The propositus was initially described as a MFS-suspected disorder together with several LDS features [Sakai et al., 2006], and is now revised as

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LDS with additional clinical findings in this report. Detailed genetic evaluation will be presented.

MATERIALS AND METHODS

DNAs

The propositus (MFS55) of the family was reported previously (Fig. 1A) [Sakai et al., 2006]. Genomic DNA was prepared from peripheral blood leukocytes of the patient and his parents using DNA extraction systems [Quick Gene-800 (Fujifilm, Tokyo, Japan) and/or NA-3000 (Kurabo, Osaka, Japan)], from nails and hair follicles of the father using an ISOHAIR kit (Nippon Gene, Toyama, Japan), and from buccal epithelial cells of the father using a Puregene Buccal Cell DNA Isolation Kit (Gentra, Minneapolis, MN). The institutional review board approved experimental protocols in this study.

Mosaic Assay

Exon 5 of *TGFBR2* was amplified by PCR with primers (F: 5′-AATCCTCTGCACGTGTCAGG-3′ and R: 5′-TGCTCGAAGCAACACATGATC-3′) using the patient's leukocyte DNA as a template. PCR products were then cloned using a TOPO-TA cloning kit (Invitrogen, Carlsbad, CA). Wild type (wt) and mutant (mt) [c.1336G > A (D446N)] clones were verified by sequencing. DNA was extracted from a wild type clone and a mutant using a QIAGEN Plasmid Midi Kit (Qiagen, Tokyo, Japan) and used as standard DNA to determine ratios of mosaicism. Wt and mt clone DNAs were mixed in different ratios: 0, 0.2, 0.4, 0.6, 0.8, and 1.2 (wt/mt). Minisequencing using a SNaPshot kit (Applied Biosystems, Foster City, CA) was performed according to the manufacturer's instruction to quantify ratios of cells with a heterozygous mutation and those without a mutation. PCR was cycled 35 times at 94°C for 30 sec, at 65°C for 30 sec, and at 72°C for 30 sec in a 20 μl mixture, containing 1× PCR buffer with 2.0 mM $MgCl_2$, 0.2 mM each dNTP, 0.5 μM each primer, 1.25 U Blend-Taq-Plus (Toyobo, Osaka, Japan) and a template DNA (30 ng of genomic DNA or 3 ng of clone DNA mixture), and treated with ExoSAP-IT (USB, Cleveland, OH) to remove primers and unincorporated deoxynucleotides. Reaction mixture consisting of 2 μl of SNaPshot ready reaction mix, 0.2 μM of extension primer [5′-NNNNNNNNNGTTGAGTCCTTCAAGCAGACC-3′ (N: random sequences)] for each nucleotide change and 2 μl of purified PCR product in a total volume of 10 μl was subjected to 25 single base extension cycles of denaturation at 96°C for 10 sec, annealing at 50°C for 5 sec and extension at 60°C during 30 sec, and treated with Calf Intestinal Phosphatase (CIP). One microliter of diluted solution was mixed with 8.5 μl of HiDi formamide (Applied Biosystems, Foster City, CA) and 0.5 μl of Gene Scan 120 LIZ Size Standard (Applied Biosystems), denatured at 95°C for 5 min, and analyzed by ABI 3100 Genetic Analyzer using GeneMapper ver 3.5 software (Applied Biosystems). Based upon mixed ratios of standard DNAs and peak areas at each SNaPshot experiment (Fig. 1D), reference curve (linear regression) was calculated (Fig. 1E). Correlation coefficient of all reference curves was larger than 0.99. Result from patient's genomic DNA from leukocytes was regarded as internal standard for 1 (wt/mt). Mosaic ratios from different sample DNAs were determined by a peak area ratio on a reference curve (Fig. 1E). Triplicated assays in one electrophoresis were repeated twice.

RESULTS AND DISCUSSION

The propositus (MFS55) with a heterozygous *TGFBR2* mutation [c.1336G > A (D446N)] was previously described as showing a MFS-suspected disorder together with several LDS features [Sakai et al., 2006]. His birth weight was 3,146 g after 38 weeks of gestation. Pregnancy and delivery were uneventful. He presented with broad and protruding forehead, deep set eyes, hypertelorism, blue sclera, bilateral strabismus, bifid uvula, malar hypoplasia, micrognathia, scoliosis, bilateral thumb camptodactyly, right talipes varus, bilateral pes planus, joint laxity of wrist and elbow, umbilical hernia, ventricular septal defect (VSD) (double committed type), bicuspid aortic valve, progressive annuloaortic ectasia and main pulmonary artery dilatation (Fig. 1A). VSD was surgically corrected at 50 days old. Craniosynostosis, high arched palate, ectopia lentis, pectus deformity, and arachnodactyly was not present. Angiography showed hypoplasia of left subclavian and vertebral arteries associated with some tortuosity, but the aorta was normal (Table I). The 3D MR angiography at age 5 years revealed the following vascular abnormalities: tortuosity of aortic arch, right brachiocephalic artery and bilateral vertebral arteries; mild dilatation of ascending aorta and right brachiocephalic artery; narrowing of bilateral vertebral arteries and bilateral subclavian artery. Ascending and abdominal aorta was normal (Fig. 1B). He is now regarded as having LDS.

Direct sequencing of *TGFBR2* in his genomic DNA clearly showed a heterozygous mutation, c.1336G > A (D446N) [Sakai et al., 2006]. Then his parental blood samples were analyzed to confirm whether the change was de novo or inherited. His mother was normal, but his father's result implied mosaicism for the mutation (Fig. 1C). Mosaicism was investigated by quantitative SNaPshot assay (Fig. 1D,E). DNA of blood leukocytes, buccal cells, hair root cells, and nails from the father indicated 52%, 25%, 0%, and 35% of cells harbored the heterozygous mutation, respectively (Table II). A complete heterozygous pattern of

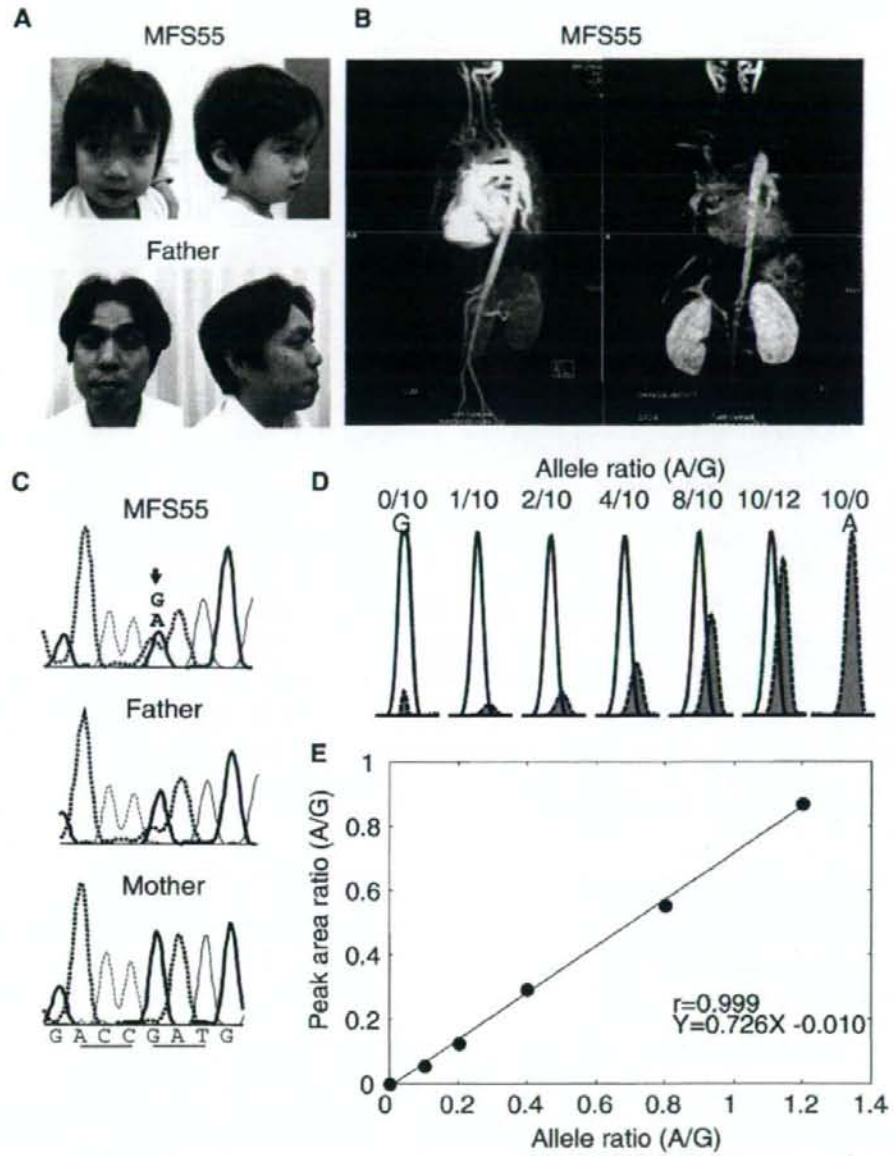

FIG. 1. **A**: Photographs of the propositus at age of 18 months and his father. **B**: 3D MR angiography of the propositus at age of 5 years. Tortuosity of aortic arch, right brachiocephalic artery and bilateral vertebral arteries, mild dilatation of ascending aorta and right brachiocephalic artery, narrowing of bilateral vertebral arteries and bilateral subclavian artery were noted. Ascending and abdominal aorta was normal. **C**: Electropherograms of the *TGFBR2* mutation, c.1336G > A in the propositus, father and mother. DNA of the peripheral blood leukocytes was analyzed. **D**: SNaPshot experiments on standard DNAs. **E**: Reference curve (linear regression) is drawn based upon mixed ratios of standard DNAs (*X*-axis) and results of SNaPshot experiments (*Y*-axis).

typical equal double peaks at the intragenic heterozygous SNP (rs1155705, *TGFBR2* IVS2 + 7A > G) was observed in DNAs of all the father's tissues examined (leukocytes, buccal cells, hair root cells and nails) according to sequencing electropherograms (data not shown). Thus observed mosaicism is specific to the mutation. Clinical examination of the father revealed that bifid uvula, narrow palate, mild