Fig. 1. The structure of *NSD1* with mutations in Sotos syndrome, Sotos-like syndrome, and other syndromes. Each box indicates exon, gray regions show functional domains (SET, PWWP, and PHD) and nuclear receptor interacting domains, NID^{-L} and NID^{+L}. Start and stop codons are located at exon 2 and 23, respectively. Arrowheads and filled circles indicate protein truncation mutations and missense mutations. Asterisks below arrowhead or circle show familial mutations. The same mutation in different individuals was shown as different arrowheads.

out of six MD cases. Among 16 PM cases, 14 were diagnosed as typical SoS and 2 as Sotos-like syndrome owing to the absence of advanced bone age. The degree of mental retardation was variable and 2 out of 16 patients had cardiac septal defect. Both reports suggested that mental retardation in SoS patients with MD is more severe than in patients with PM and cardio-vascular complications in SoS patients with MD are more frequent than in those with PM.

NSD1 Mutations in Other Overgrowth Syndromes

Notably, in six cases of Weaver syndrome, whose phenotype overlaps significantly with SoS *(17,18,37)* and in two cases of another overgrowth syndrome, Beckwith-Wiedemann syndrome (BWS), *NSD1* mutations were identified *(18)*. In Weaver syndrome, 6 cases out of 13 had *NSD1* intragenic PM *(17,20)*, suggesting that SoS and Weaver syndrome are allelic. The majority of BWS is caused by either genetic alterations (11p15 paternal uniparental disomy or *CDKN1C* mutations) or epigenetic defects (demethylation of the *KvDMR1* region of *KCNQ1OT* and hypermethylation of *H19*) *(38–40)*. Interestingly, in addition to two BWS cases with *NSD1* mutations, two SoS cases without *NSD1* abnormality showed abnormal status of the 11p15 region (demethylation of *KCNQ1OT* and a paternal isodisomy of 11p15) *(18)*. These data indicate challenges for proper clinical diagnosis of even well-established overgrowth syndromes. Another possibility is an unknown common pathway among the three syndromes. It is very important to evaluate *NSD1* status in other overgrowth syndromes to elucidate whether *NSD1* mutations are specific not only for SoS.

IS SOS A GENOMIC DISORDER?

Fifty MDs have been analyzed using fluorescence *in situ* hybridization and microarray comparative genomic hybridization (4). Three different types of microdeletions were delineated, among which, the approx 2-Mb MD I (Fig. 2A) was the most common (found in 46 out of 50 patients). In the other four cases two smaller MDs were recognized. Highly homologous regions at each deletion breakpoints of the MD I were identified (4–6). These LCRs were termed Sotos syndrome distal-repeat (SoS-DREP, approx 429 kb) and proximal-repeat (SoS-PREP, approx 390 kb) (Fig. 2). Sequence comparisons of SoS-DREP and SoS-PREP revealed that six sequence homology subunits (A–F) of PREP showed more than 96% identity to DREP (Fig. 2B). Their sizes of SoS-PREP subunits were 123.6 kb (A), 20.1 kb (B), 62.8 kb (C), 7.8 kb (D), 8.2 kb (E), and 93.9 kb (F) and those of SoS-DREP subunits were 119.1 kb (A), 19.7 kb (B), 68.7 kb (C), 7.8 kb (D), 8.3 kb (E), 82.8 kb (F), and 50.1 kb (C'). Each of the homologous subunits, with the exception of one, is located in an inverted orientation and the order of subunits is different between the two SoS-REPs. Only the subunit C' in SoS-DREP is oriented directly with respect to the subunit C in SoS-PREP. These subunits are more than 99% identical. Two recent reports showed that the subunit C' in SoS-DREP and the subunit C in SoS-PREP, were utilized as a substrate of NAHR of the SoS common deletion (5,6). In addition, the reports indicated that the crossover events occurred in those subunits and that an approx 80% of crossover hotspots were within an approx 3-kb genomic sequence in those subunits (5,6) (Fig. 2).

These data established that SoS is a new genomic disorder and an NAHR mechanism is a consistent mechanism for generation of the SoS common deletion as in other genomic disorder reported (41–43).

IS SOS A CONTIGUOUS GENE SYNDROME?

There are at least 22 genes that map within the common deleted region (UCSC Genome Browser, May 2004 Assembly, http://genome.ucsc.edu/cgi-bin/hgGateway) (Fig. 2). Both SoS-REPs contain two genes, *THOC3* and *NY-REN-7* (Fig. 2). *THOC3* and *NY-REN-7* have open reading frames that are completely conserved in SoS-PREP and SoS-DREP. The *PROP1* gene maps only to SoS-DREP between subunit E and C' (Fig. 2A). Among those 22 genes deleted, *NSD1*, the plasma coagulation factor 12 gene (*F12*, OMIM +234000), the prophet of the *PIT-1* gene (*PROP1*, OMIM +601538), and the xylosylprotein β 1,4-galactosyltransferase, polypeptide 7 gene (*B4GALT7*, also known as xylosylprotein 4-β-galactosyltransferase I gene, *XGTP1*, OMIM *604327) may be directly related to human phenotypes.

F12 encodes the coagulation factor XII, also known as Hageman factor. Heterozygous deletion of *F12* may result in partial F12 deficiency, which could present with a slight to moderate bleeding tendency (44,45). Low levels of factor XII activity may also be a risk factor for repeated spontaneous abortions or skin ulcers (46,47). A common polymorphism in the 5'-untranslated region of *F12*, the c.46C > T substitution, was found to be associated with low F12 level (48). In cases of c.46T/T, the value of F12 was remarkably decreased. Soria et al. (49) reported that the 5q33-qter region is a quantitative risk factor for thrombosis using genome wide linkage analysis. A novel homozygous p.W484C mutation was shown to induce low F12 levels (50). It is important to evaluate F12 in SoS patients with MDs, although such a risk has not been known in SoS.

Homozygous or compound-heterozygous defects of *PROP1* result in combined pituitary hormone deficiency including GH, PRL, TSH, LH and FSH (OMIM +601538) (51). So far,
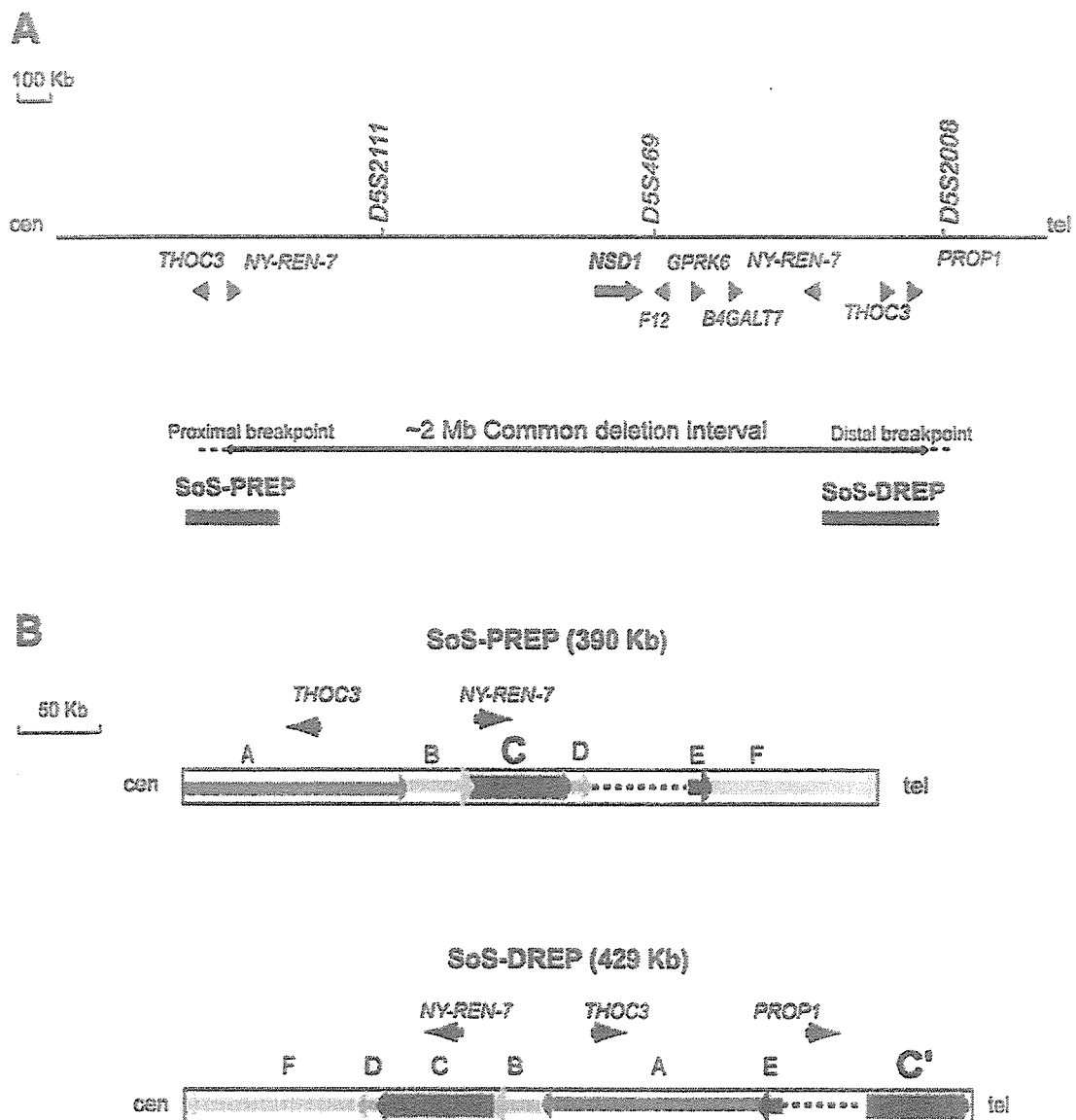
Fig. 2. (A) Physical map depicting microdeletions found in Sotos syndrome (SoS) and two low-copy repeat sequences, termed proximal-repeat (SoS-PREP) and distal-repeat (SoS-DREP) at 5q35. The Sos-REPs, indicated as black boxes, are proximal and distal to *NSD1*. Among 22 genes that map within the deletion interval, *NSD1*, the SoS-REP-specific predicted genes (*THO3, NY-REN-7*, and *PROP1*), and possible human phenotype-related genes (*F12, GPRK6, B4GAL4T7*) are presented. *THOC3* and *NY-REN-7* map to both SoS-PREP and SoS-DREP. Bold bi-directional arrow represents a deleted region. An approx 2-Mb microdeletion is the most commonly observed in SoS. (B) There are six subunits of more than 96% sequence identity between the proximal and the distal SoS-REPs (A–F); their orientation is depicted as arrow. All subunits except C' are inverted with respect to each other. Dotted lines indicate unique sequence in low-copy repeats. Three relevant genes are shown.

three SoS cases associated with hypothyearsoidism have been reported *(52,53)*. Unmasking of the recessive allele is possible when one allele harbors a PM and the other is deleted. It may be worth investigating *PROP1* if hypothyearsoidism is observed.

B4GALT7 regulates the synthesis of various glycosaminoglycans (GAGs). GAGs are basic components of heparin/heparan sulfate or those of chondroitin sulfate/dermatan sul-

fate and have an important role in the formation of various tissues and organs *(54)*. Defects of GAGs may be possibly responsible for the various forms of so-called mucopolysaccharidoses. In the progeroid type of Ehlers-Danlos syndrome, compound heterozygosity for p.A186D and p.L206P mutations of *B4GALT7* was confirmed. The father was heterozygous for the p.L206P allele and mother heterozygous for the p.A186D allele *(55,56)*. Although only one case with such mutations has been reported, carrier status for such PMs in contributions with hemizygous deletion of *B4GALT7* in SoS patients with MDs could contribute to phenotypic variability.

GPRK6 encodes G protein-coupled receptor kinase 6 protein (GPRK6) (OMIM *600869), which can regulate G protein-coupled receptors. Using immunohistochemistry, GPRK6 expression was confirmed in striatal neurons receiving dopaminergic input and postsynaptic D2/D3 dopamine receptors were targets of GPRK6 *(57)*. Investigation of *GPRK6* by gene targeting to create a knockout animal shows higher sensitivity to psychostimulants including cocaine and amphetamine especially in homozygous mice rather than heterozygous, suggesting that such high sensitivity may be related to some potential psychiatric diseases in human *(58)*. It would be interesting to evaluate for different psychiatric and behavioral aspects between SoS cases with MD versus PM.

The influence of the deletion of 21 genes other than *NSD1* needs to be carefully evaluated, as some genes may affect the severity of phenotypes in MD patients.

FUTURE DIRECTION

Rearrangement-prone regions of the human genome including LCRs have been challenging to sequence *(59,60)*. Validation and mapping of MD breakpoints at the nucleotide level should provide further insights into the mechanisms of DNA rearrangement. Functional studies of *NSD1* are required for elucidating pathophysiological aspects of SoS. Intensive molecular analyses of 282 patients with SoS, Sotos-like syndrome, and Weaver syndrome revealed the *NSD1* abnormalities in 168 cases; *NSD1* was intact in the other remaining 114 cases. Improved methods to detect other types of *NSD1* abnormalities, including partial deletion and nucleotide changes of introns and promoter regions, and more data of clinical phenotypes observed in patients with MDs and PMs should reveal further genotype/phenotype correlations and provide insights into SoS pathophysiological mechanisms.

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Nevo Syndrome With an *NSD1* Deletion:
A Variant of Sotos Syndrome?

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A 17-month-old girl with clinical manifestations of Nevo syndrome and *NSD1* (nuclear receptor binding SET domain protein 1) deletion is described. Nevo syndrome is a rare overgrowth syndrome showing considerable phenotypic overlap with Sotos syndrome—another, more frequent overgrowth syndrome caused by *NSD1* mutations or deletions. About a half of Japanese Sotos syndrome patients carry a 2.2-Mb common deletion encompassing *NSD1* and present with frequent brain, cardiovascular, or urinary tract anomalies. The girl we described had the common deletion and showed patent ductus arteriosus, atrial septal defect, vesicoureteral reflux, and bilateral hydronephrosis. It was thus concluded that the clinical manifestations, including the Nevo syndrome phenotype, were caused by the microdeletion. © 2005 Wiley-Liss, Inc.

Key words: Nevo syndrome; Sotos syndrome; overgrowth; *NSD1*; microdeletion

INTRODUCTION

Nevo syndrome is a rare overgrowth syndrome characterized by joint laxity, kyphosis, wrist drop, spindle shaped fingers, and volar edema, and shares many phenotypic features with Sotos syndrome [Nevo et al., 1974]. Sotos syndrome is characterized by overgrowth with advanced bone age, a dysmorphic face with macrocephaly, large hands and feet, and mental retardation [Cole and Hughes, 1994]. Recently, a 2.2-Mb microdeletion encompassing the *NSD1* gene was reported in a series of Japanese patients with Sotos syndrome [Kurotaki et al., 2003]. Patients with an *NSD1* deletion tend to have anomalies in the central nervous system (e.g., agenesis or hypoplasia of the corpus callosum), cardiovascular system (e.g., patent ductus arteriosus and atrial septal defect (ASD)), and urinary system (e.g., vesicoureteral reflux, hydronephrosis, and small kidney) [Nagai et al., 2003]. We described a young girl with Nevo syndrome with an *NSD1* deletion, and presenting with both cardiac and urinary abnormalities in addition to volar edema, and contractures of hands and feet, which are typical and pivotal features of Nevo syndrome. We postulated that the clinical manifestations in the girl, including the Nevo syndrome phenotype, were due to the microdeletion.

CLINICAL REPORT

The girl was born at full-term after an uneventful pregnancy as the first child of non-consanguineous parents. The 22-year-old father measured 170 cm and weighed 70 kg, the 19-year-old mother was 160 cm and 79 kg. Birth weight of the girl was 3,450 g (+0.4 SD), length 52 cm (+1.6 SD), and OFC 35 cm (+1.5 SD). She showed flexion contractures of hands and feet, valgus deformity of the right foot, and

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muscular hypotonia. Hyperbilirubinemia was treated with phototherapy for a few days. Growth, especially weight gain, was extremely accelerated. She sat alone at age 10 months and stood supported at 15 months. At age 17 months, her height was 87 cm (+3 SD), weight 13.8 kg (+3.6 SD), and OFC 48.5 cm (+1.8 SD). Motor and speech development were delayed. Although cognitive function was normal at age 17 months, she could not speak a word. When examined by us at age 17 months, generalized hypotonia and thoracic kyphosis were present. Her craniofacial features included dolichocephaly, a narrow high-arched palate, large abnormal low-set ears, and a webbed neck (Fig. 1a,b). The hands and feet were large and edematous. In addition, there was wrist drop and spindle shaped fingers (Fig. 1c,d). Echocardiography demonstrated an ASD and patent ductus arteriosus (PDA). Computed tomography of the brain showed no structural deformity. Bilateral hydronephrosis was seen on ultrasonography, and voiding cystoureterography revealed bilateral vesicoureteral reflux. Chromosome analysis revealed a normal 46,XX karyotype. At age 15 months, her bone age was advanced being 30 months, particularly seen in the metacarpophalangeal bones. Radiographic abnormalities included 11 pairs of thin ribs with a left cervical rib, thoracholumbar scoliosis, mild iliac hypoplasia, mild undermodeling of the proximal femora, and large epiphyses of the hip and knee bones.

We performed chromosomal and interphase FISH analysis on cultured peripheral blood lymphocytes from the patient using seven BAC/PAC clones encompassing *NSD1*, which detect deletions or mutations leading to Sotos syndrome [Kurotaki et al., 2003]. Five (RP11-349N15, RP11-1006E8, RP11-606E24, RP1-118M12, and RP11-147K7) of the seven clones showed a deletion in one of homologous chromosomes 5, whereas the remaining two clones (RP11-355H1 and RP11-158F10) never showed deletion (Fig. 2). Her deletion thus involved a 2.2-Mb region encompassing *NSD1* with its proximal breakpoint between RP11-355H1 and RP11-349N15, and its distal breakpoint between RP11-147K7 and RP11-158F10.

DISCUSSION

The girl we have described had clinical manifestations common to both Nevo and Sotos syndromes,

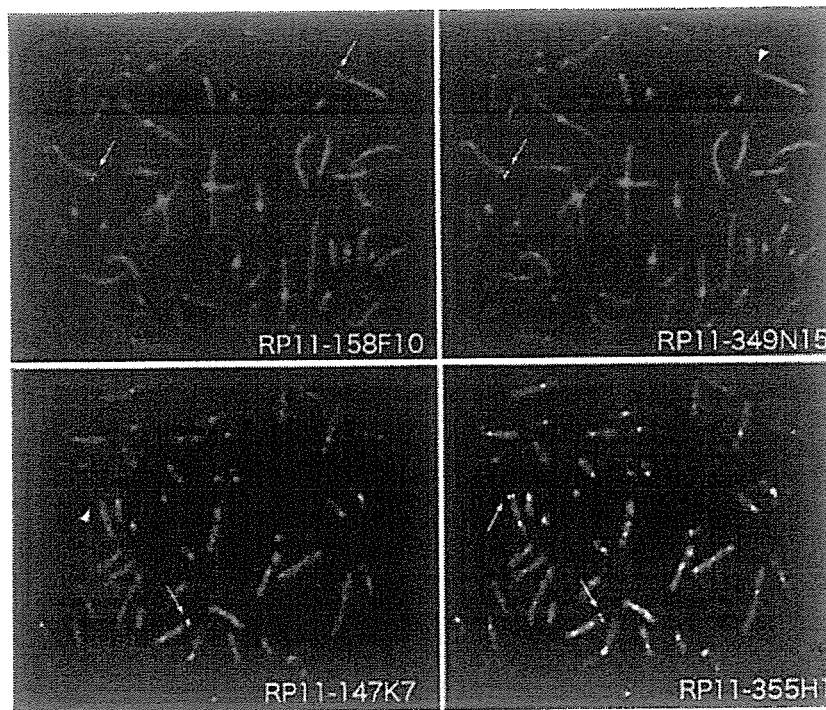Fig. 1. The patient. (a) at age 9 months. b, c, and d: at age 17 months.

Fig. 2. FISH analysis of metaphase chromosomes from the patient. RP11-158F10 and RP11-355H1 are present (arrows), whereas RP11-349N15 and RP11-147K7 are deleted (arrowheads).

TABLE I. Phenotypic Comparison of Present and Other Patients With Nevo Syndrome or Sotos Syndrome With *NSD1* Deletions

Clinical manifestations	Present patients	Patients with	
		Nevo syndrome[a]	Sotos syndrome with deletions[b]
Common to both syndromes			
Neonatal hyperbilirubinemia	+	4/7	11/21
Retarded motor and speech development	+	2/7	9/9
Overgrowth	+	7/7	18/21
Advanced bone age	+	5/7	10/14
Coarse facial appearance	+	7/7	20/20
Prominent forehead	+	7/7	20/20
Large, low-set ears	+	6/7	16/20
High palate	+	6/7	17/18
Large hands and feet	+	5/6	20/21
Frequently seen in Sotos syndrome with deletion			
Brain anomalies	−		12/19
Cardiovascular anomalies	+[c]		12/21
Urinary tract anomalies	+[c]		7/13
Frequently seen in Nevo syndrome			
Generalized hypotonia	+	7/7	7/21
Normal head circumference	+	3/4	2/20[d]
Kyphosis	+	7/7	
Scoliosis	+	3/7	5/21
Cryptorchism		6/6	
Edema of hands and feet	+	6/7	
Wrist drop	+	7/7	
Spindle shaped fingers	+	6/6	
Valgus deformity of the feet	+	3/3	

[a]Data from Nevo et al. [1974], Hilderink and Brunner [1995], al-Gazali et al. [1997], and Dumic et al. [1998].
[b]Data from Nagai et al. [2003].
[c]PDA, ASD, vesicoureteral reflex, and hydronephrosis.
[d]Macrocephaly in 18/20 cases.

i.e., those frequently seen in Sotos syndrome patients with deletions encompassing *NSD1*, and those frequent or unique in Nevo syndrome patients (Table I). Nevo syndrome is a childhood overgrowth syndrome that shares many phenotypic features with Sotos syndrome. Identification of a common *NSD1* deletion in our patient strongly suggests that the two syndromes are either allelic. Alternatively, they may causally be related each other. In the latter case, Nevo syndrome is assumed to be an autosomal recessive disorder, and a mutant allele of the putative gene on the non-deleted chromosome 5 may be expressed through the unmasking heterozygosity mechanism by the deletion. Of seven previously reported patients with Nevo syndrome, three were two brothers and their male cousin from an inbred Arab family [Nevo et al., 1974], and two other patients were from two unrelated Arab families from different tribes [al-Gazali et al., 1997]. A male patient with the syndrome from the Netherlands was born to consanguineous parents [Hilderink and Brunner, 1995]. These findings may indicate a concentration of the syndrome among the Arab population, and support its autosomal recessive inheritance. It remains to be seen whether Nevo syndrome patients have *NSD1* mutations.

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Clinical Report

A Large Interstitial Deletion of 17p13.1p11.2 Involving the Smith–Magenis Chromosome Region in a Girl With Multiple Congenital Anomalies

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A 6-month-old girl had multiple congenital anomalies, including dysmorphic face; tetralogy of Fallot, pulmonary atresia and patent ductus arteriosus; congenital cystic adenomatoid malformation of the right upper lung, and hemilateral kidney defect. Chromosome analysis as well as flurorescence in situ hybridization (FISH) and polymorphic marker analyses in the girl and her parents revealed a de novo large interstitial deletion of 17p13.1-p11.2 of the paternally derived chromosome 17. The deletion involved the Smith–Magenis chromosome region (SMCR). Lack of involvement of the Miller–Dieker syndrome region at 17p13.3 was confirmed by both FISH analysis and radiological examinations that showed no migrational abnormality. The girl died at age 7 months. This is the first report of a patient with a large interstitial deletion of 17p. © 2005 Wiley-Liss, Inc.

Key words: 17p11.2; Smith–Magenis syndrome; large interstitial deletion; dysplastic kidney; cyanotic congenital heart disease; fluorescence in situ hybridization (FISH)

INTRODUCTION

There are two major contiguous gene syndromes associated with 17p, Miller–Dieker syndrome (MDS, OMIM #247200) and Smith–Magenis syndrome (SMS, OMIM #182290), both of which have been well studied. MDS involves a subtelomeric deletion of 17p13.3 [Mutchinick et al., 1999; Cardoso et al., 2003]. Reiner et al. [1993] cloned the *LIS1* (lissencephaly-1) gene at 17p13.3, which is deleted in MDS patients. Subsequently, many single-mutations in this gene were reported to be the cause of isolated lissencephaly [Lo Nigro et al., 1997]. SMS is an interstitial deletion syndrome of 17p11.2. The mechanism of formation for this interstitial deletion is thought to be non-allelic homologous recombination between low-copy repeats (LCR) [Shaw and Lupski, 2005]. The deleted regions of SMS are usually common among patients [Shaw et al., 2004]. Slager et al. [2003] identified mutations in the *RAI1* gene, and now haploinsufficiency of *RAI1* is believed to be respon-

sible for the behavioral, neurologic, otolaryngologic, and craniofacial abnormalities of SMS. More variable features, such as heart and renal defects, are probably due to hemizygosity of other genes involved in the 17p11.2 deletion.

Recently, we encountered a girl who had a large interstitial deletion of 17p, including the Smith–Magenis chromosome region (SMCR). To our knowledge, this is the largest deletion of 17p in the literature. Here we describe the clinical details of this patient.

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CLINICAL REPORT

A 29-year-old, primigravida woman was referred to us at 35 weeks of pregnancy because of intra-uterine growth retardation of the fetus. The woman and her 31-year-old husband were healthy and non-consanguineous. Tetralogy of Fallot (TOF) and congenital cystic adenomatoid malformation (CCAM) of the right upper lung in a fetus were suspected by prenatal ultrasonography. Caesarean was performed at 38 weeks of gestation because of breech presentation. The baby, a girl, weighted 1,978 g (−2.9 SD), and had length of 41.8 cm (−3.8 SD) and OFC of 30.6 cm (−1.7 SD). Echocardiography confirmed TOF with coarctation of the left pulmonary artery, and patent ductus arteriosus (PDA). Continuous injection of prostaglandin E1 (PGE1) was started to keep the duct open. At age 72 days, the girl received modified right Blalock-Taussig shunting operation with a Gore-Tex graft (3 mm), and her left pulmonary artery was reconstructed. Branch artery from aorta descendens was detected by chest computed tomography (CT), which confirmed CCAM of the right upper lung. Abdominal echography showed agenesis of the left kidney.

Brain MRI at age 2 months showed mild dilatation of bilateral cerebral ventricles; hypoplasia of the white matter with delayed myelination, the cerebellar vermis and of the corpus callosum, but no migrational abnormality in the cerebral cortex (Fig. 1B,D). At age 6 months, she was fed through a nasogastric tube. She followed moving objects, but instability of her neck persisted. She had upslanting palpebral fissures, low-set ears, low nasal bridge, micrognathia (Fig. 1A), a cleft palate, nuchal skin folds, a single flexion crease of the left fifth finger, bilateral single palmar creases, overlapping toes, rocker-bottom feet, sacral bossing, and a sacral dimple. She died at age 7 months of cardiac failure. Autopsy was not granted.

MOLECULAR AND CYTOGENETIC STUDIES

G-banded karyotyping of cultured peripheral blood lymphocytes showed a large deletion at 17p (Fig. 1E). Fluorescence in situ hybridization (FISH) analysis by the use of LSI SMS (Vysis, Downers Grove, IL) demonstrated a deletion of the SMCR (Fig. 2A), while that with LSI LIS1 (Vysis) showed no deletion of *LIS1* (Fig. 2B). Detailed FISH analysis on interphase nuclei of the patient using BAC clone probes mapped to 17p13.3-11.2 as described elsewhere [Shimokawa et al., 2004] showed a deletion of 17p13.1-11.2 (Table I). Thus, her karyotype is interpreted as 46,XX,del(17)(p13.1p11.2).ish del(17)(LIS1+, RP1-95H6+, RP11-545O6+, RP11-457I18+, RP11-61B20+, RP11-89A15+, RP11-746E23−, RP11-125H11−, RP11-270I9−, RP11-385G5−, RP11-746E8−, RAI1−, RP1-172N16−,
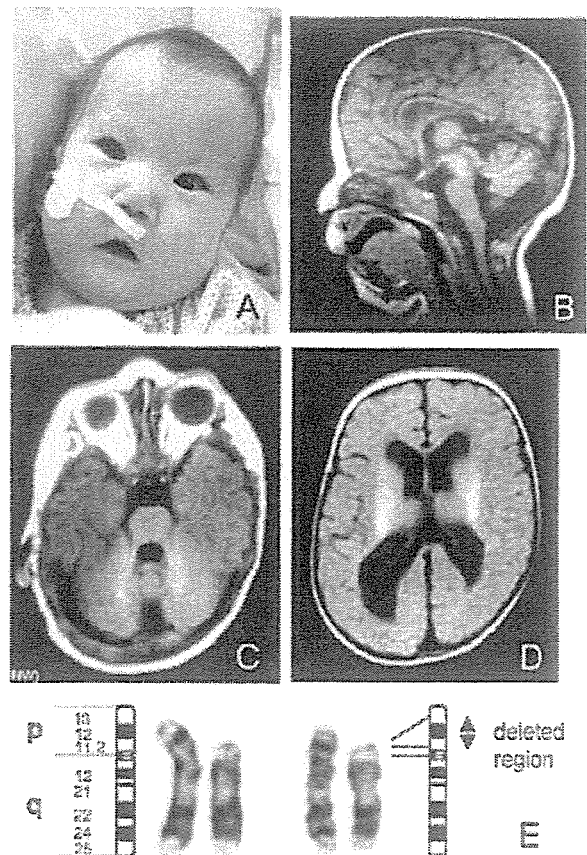

FIG. 1. Facial dysmorphology of the patient at age 4 months (A), including upslanting palpebral fissures, low-set ears, low nasal bridge, and micrognathia. MRI with T1W view of sagittal (B) and axial (C,D) sections at different levels, showing hypoplasia of the cerebellar vermis and corpus callosum (B), mild dilatation of bilateral cerebral ventricles with white matter hypoplasia and delayed myelination. No migrational abnormality is seen in the cerebral cortex (D). Molar tooth sign is absent (C). G-banded chromosome 17 from the patient and corresponding schematic representation (E). A large deletion of 17p is indicated in each right-sided chromosome.

RP11-160E2−, RP11-363P3−, RP11-64J19−). Karyotypes of both parents were normal.

Haplotype analysis of this family was performed using the following microsatellite markers: D17S969, D17S1296, D17S2186, D17S1356, D17S1357, GATA185H04, and D17S130. Primer information was retrieved from the in-silico library [Cooperative Human Linkage Center (http://gai.nci.nih.gov/CHLC/) and UCSC Genome Bioinformatics Site (http://genome.ucsc.edu/)]. Genomic DNA was obtained from the patient and her parents, and subsequent PCR amplification was performed according to the standard method. The amplicons were visualized by ethidium bromide staining after separation by electrophoresis on an acrylamide gel. Only the result from D17S969 (located at 17p12) was informative for detecting the parental origin of the deletion. As the patient had a band for this marker in common only with her mother (Fig. 2C), it was deduced that the
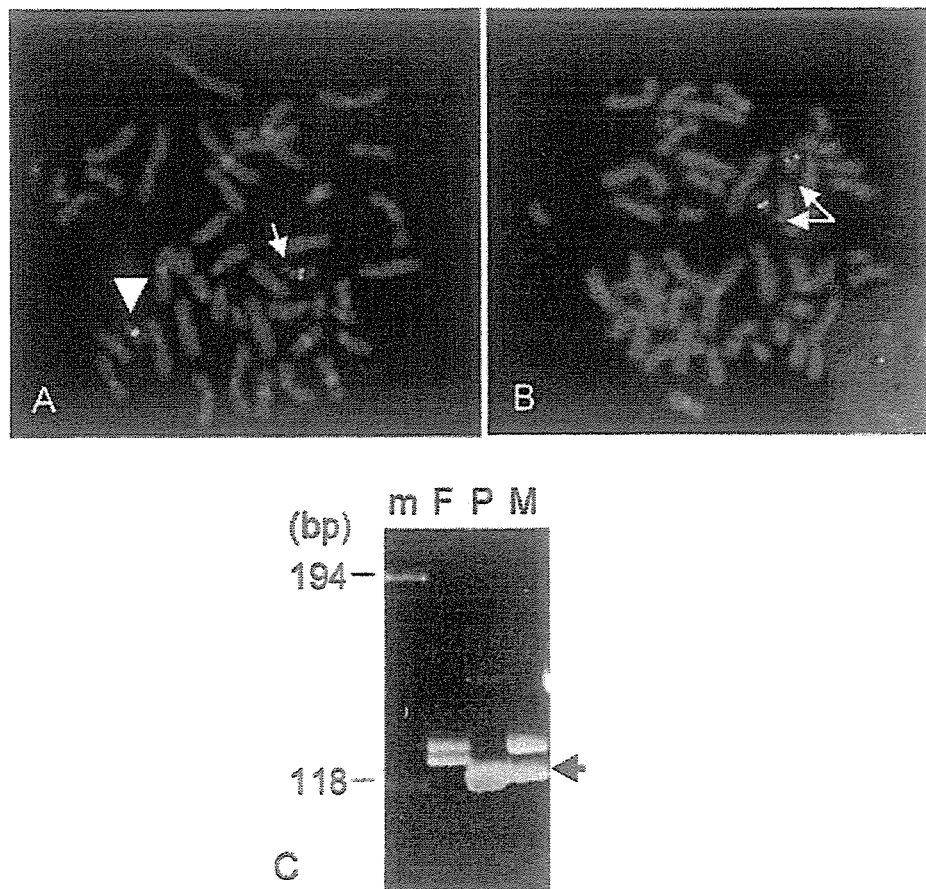
Fig. 2. Two-color FISH analysis of the patient's chromosomes using LSI SMS Spectrum Orange/RARA Spectrum Green dual-color DNA probe (A). Green signals were obtained by LSI RARA SpectrumGreen at band 17q21. Arrow and arrowhead indicate the presence and absence, respectively, of a signal for the SMCR. Detection of the *LIS1* region using SpectrumOrange LSI LIS1 at band 17p13.3 (B). Arrows indicate the presence of the *LIS1* signal. Paternal origin of the deletion (C). The patient inherited only the maternal allele (arrow). Electrophoretic bands of PCR products for microsatellite marker, D17S969, of the patient and her parents were visualized by ethidium bromide staining. m, marker (ϕX174/*Hae*III digest); F, father; P, patient; M, mother. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

TABLE I. BAC Clones Located to 17p13.1-11.2 Used as Probes in FISH Study

| | Location | | | | |
| | Chromosome band* | Nucleotide position | | Disease locus | Result of FISH |
Clone name		Start*	End*		
RP1-95H6	17p13.3	2443685	2535638	*LIS1*	Normal
RP11-545O6	17p13.2	3550805	3735942		Normal
RP11-457I18	17p13.2	5093178	5301854		Normal
RP11-61B20	17p13.1	6780963	6943107		Normal
RP11-89A15	17p13.1	8199810	8365717		Normal
RP11-746E23	17p13.1	8576926	8748280		Deletion
RP11-125H11	17p13.1	9176082	9364840		Deletion
RP11-270I9	17p13.1	9745074	9904704		Deletion
RP11-385G5	17p12	11132724	11344170		Deletion
RP11-746E8	17p12	12435731	12620614		Deletion
RP11-172N16	17p11.2	18069802	18201100	*SMS*	Deletion
RP11-160E2	17p11.2	18863814	19021902		Deletion
RP11-363P3	17p11.2	20012562	20185625		Deletion
RP11-64J19	17p11.2	21014522	21191548		Deletion

*Chromosomal location or nucleotide position are from UCSC database (May 2004).

paternally derived allele at this locus was deleted in the patient.

DISCUSSION

The girl described here had a de novo large interstitial deletion of 17p13.1-11.2, including the SMCR, on the paternally derived chromosome 17. The lack of clinical manifestations for MDS, including lissencephaly was confirmed by molecular cytogenetic analysis, that is, the *LIS1* region, which is critical for MDS, was intact. Hereditary neuropathy with liability to pressure palsies (HNPP) can be caused by a deletion of the *PMP22* (peripheral myelin protein-22) gene that should be included in her deletion extent. However, since the age of onset of HNPP is usually between 15 and 20 years, she was too young to present the symptoms for the disease such as neuropathy.

The patient had facial dysmorphism showing a broad, flat nasal bridge; upslanting palpebral fissures, sparse eyebrows, broad midface, and cleft palate. These manifestations are characteristic of typical of SMS [Greenberg et al., 1991]. Other facial anomalies such as a long philtrum and a carp-shaped mouth, and hemilateral dysplasia of the kidney have not been reported in SMS patients. It is likely that these abnormalities were due to hemizygosity of genes other than those in the SMCR. Severe congenital heart disease (CHG) of the patient, including TOF, PA, and VSD, is also compatible with SMS. Greenberg et al. [1996] described that at least 37% of SMS patients had CHG, and some patients were reported to have TOF, PA, and VSD [Smith et al., 1986; Wong et al., 2003; Myers and Challman, 2004]. Thus, the heart anomalies in our patient are not exceptional for SMS. Natacci et al. [2000] described a patient who had SMS associated with Joubert syndrome. Our patient also had hypoplasia of the cerebellar vermis but molar tooth sign was not identified by MRI. Thus, Joubert syndrome was ruled out in our case.

In conclusion, this is, to our knowledge, the first report of a large deletion of 17p. From our experience with this patient, such a deletion may lead to a poor prognosis, early infantile lethality. Our case may contribute to better understanding of a genotype–phenotype correlation of SMS.

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Research Letter
No Detectable Genomic Aberrations by BAC Array CGH in Kabuki Make-Up Syndrome Patients

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To the Editor:

Kabuki make-up syndrome (KMS, OMIM 147920), independently established by Niikawa et al. [1981] and Kuroki et al. [1981], is characterized by characteristic facial features resembling the Kabuki actor's make-up, mild to moderate mental retardation, postnatal growth retardation, skeletal abnormalities, and unusual dermatoglyphic patterns [Matsumoto and Niikawa, 2003]. The multisystem involvement of the KMS phenotype suggests that KMS is caused by a microdeletion or microduplication involving several genes. Milunsky and Huang [2003] reported that all of the six KMS patients they examined had approximate 3.5-Mb duplication at 8p22–p23.1 revealed by comparative genomic hybridization (CGH) and fluorescence in situ hybridization (FISH). They also suggested that a paracentric inversion in mothers, detected by RP11-122N11, might contribute to the occurrence of the

syndrome. At least three groups, including us, failed to replicate their results by FISH and/or array CGH analysis [Miyake et al., 2004; Engelen et al., 2005; Hoffman et al., 2005]. Schoumans et al. [2005] reported that they observed no chromosomal abnormalities in 10 affected Caucasian individuals with typical KMS using the 1.2-Mb-resolution whole genome BAC array.

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Using a newly developed BAC array covering the whole genome with a 1.5-Mb resolution, in this study, array CGH (aCGH) was performed in 38 patients with typical KMS to find genomic imbalances in relation to KMS. Detailed experimental protocols are described elsewhere [Miyake et al., 2005]. Peripheral blood leukocytes or lymphoblastoid cell lines from the 38 patients (20 males and 18 females) and their parents when available were sent to us after informed consent. All KMS cases were sporadic and had a normal karyotype according to G-banded chromosomal analysis at the 400-band level. Their metaphase chromosomes for FISH and DNA for aCGH were prepared either from immortalized lymphoblastoid cell lines or peripheral blood leukocytes according to standard protocols.

We detected a total of 115 clones implying occult genomic imbalances, which were all subsequently confirmed by FISH (14 clones as deletions and 101 clones as duplications) (Fig. 1). Heterozygous deletion/duplication might be associated with the pathogenesis of KMS, because an autosomal (or pseudoautosomal) dominant inheritance is suspected in KMS [Matsumoto and Niikawa, 2003]. All copy number changed loci (clones) observed in this

series of KMS patients, except four loci, were unlikely to be associated with KMS, because the same changes were also observed in either of their healthy parents, and/or non-KMS individuals with non-syndromic mental retardation [Miyake et al., 2005], and/or were registered in the Database of Genomic Variants (http://projects.tcag.ca/variation/). One heterozygous deletion at RP11-97F19 (2p11.2) in two patients (KMS5 and KMS17), and two heterozygous duplications at RP11-418N20 (Xp22.33) and RP4-617A9 (Xp22.3) in a patient (KMS14) remain inconclusive, because parental samples were unavailable. These changes in the three patients are described in detail below. Among 37 loci showing copy number changes in KMS patients, a total of 11 loci were already registered as copy number polymorphisms (CPNs) in the Database of Genomic Variants (checked on July 19, 2005) (Fig. 1), confirming that these changes are unlikely pathogenic.

RP11-97F19 Deletion at 2p11.2 in KMS5 and KMS17

We delimited the deletion to an approximate 1-Mb region from RP11-15J7 to RP11-136K15 by detailed

Fig. 1. Summary of BAC array CGH and FISH studies on KMS patients. Top yellow row indicates KMS patients (male in black, and female in red). Left green columns show information of clone IDs and their chromosomal locations. Duplications are shown as in red (homozygous) and orange (heterozygous). Deletions are in pale-blue (heterozygous) and dark-blue (hemizygous). Parental origin of changes is indicated as P (paternal) and M (maternal). No de novo changes were confirmed. Y in right column indicates clone/locus registered as copy number variation (CPN) locus in the Database of Genomic Variants (http://projects.tcag.ca/variation/). N clone/locus not registered in the database.

FISH analysis [UCSC Genome Browser NCBI build 35 (May 2004) coordinates, chromosome 2 nucleotide 88979594–89962288 bp] (data not shown). The gain of this region was reported previously [Sebat et al., 2004] and is described at the Database of Genomic Variants (http://projects.tcag.ca/variation), but the loss has never been reported. We did not find any cases with the same deletion in 200 chromosomes of normal Japanese controls. Regarding the duplication, homozygous and heterozygous duplication were found in 92 and 8 controls, respectively. The allele frequencies of the duplication in KMS and normal controls were 87.5% and 96%, respectively. No established genes exist within the deletion.

RP4-617A9 and RP11-418N20 Duplication at Xp22.3 in KMS14

RP4-617A9 and RP11-418N20 are closely located ~0.12 Mb apart. The heterozygous duplication of RP4-617A9 and RP11-418N20 in KMS14 was observed by aCGH. FISH analysis revealed that the duplication spans about 0.7 Mb from RP11-794A12 (distal) to RP11-418N20 (proximal) (UCSC coordinates, chromosome X nucleotide 2341315–3106243). None of 98 chromosomes in normal Japanese controls possessed the duplication. In addition, the gain of this region has not been reported yet at Database of Genome Variants. This region was overlapped with a part of the pseudoautosomal region 1 (PAR1). Among seven genes mapped to the duplication, ZBED1 and CD99 were in PAR1. Though they are attractive candidate genes according to a pseudoautosomal dominant inheritance hypothesis [Matsumoto and Niikawa, 2003], we could only find three SNPs in CD99 [68A > G (D23G), 496A > G (M166V), 518A > T (N173I)], but no pathological nucleotide changes of the two genes in 37 other KMS patients (data not shown).

In conclusion, our study of 38 KMS patients did not show any pathological copy number changes, similar to the previous report [Schoumans et al., 2005]. Thus, it is less likely that microdeletions/duplications are frequent pathological changes in KMS. KMS may be caused by defects of a single gene that regulates various target genes/organs.

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BAC Array CGH Reveals Genomic Aberrations in Idiopathic Mental Retardation

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Array using 2,173 BAC clones covering the whole human genome has been constructed. All clones spotted were confirmed to show a unique signal at the predicted chromosomal location by FISH analysis in our laboratory. A total of 30 individuals with idiopathic mental retardation (MR) were analyzed by comparative genomic hybridization using this array. Three deletions, one duplication, and one unbalanced translocation could be detected in five patients, which are likely to contribute to MR. The constructed array was shown to be an efficient tool for the detection of pathogenic genomic rearrangements in MR patients as well as copy number polymorphisms (CPNs).
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Key words: mental retardation; BAC array CGH; FISH; chromosomal abnormality

INTRODUCTION

Mental retardation (MR) occurs with the prevalence of 2%~3% of general population [Knight et al., 1999]. Chromosomal rearrangements at subtelomeric regions have been detected in 5%~6% of affected individuals with MR [Flint et al., 1995; Knight et al., 1999; de Vries et al., 2001, 2003; Harada et al., 2004], and the whole genome array comparative genomic hybridization (aCGH) with an approximate 1-Mb resolution detected pathological genomic imbalances in 14%–20% of MR cases [Vissers et al., 2003; Shaw-Smith et al., 2004], implying that aCGH is an

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essential method to detect submicroscopic chromosomal abnormalities in patients with idiopathic learning disabilities.

We have developed a new BAC array system covering the whole genome with a 1.4-Mb resolution after eliminating problematic clones with multiple and/or erroneous FISH signals in our laboratory. Thus all clones used for this array were confirmed to show a unique signal at a predicted chromosomal position. Using this system, aCGH was performed in 30 idiopathic MR to validate genomic imbalances in relation to MR. Several chromosomal abnormalities as well as copy number polymorphisms (CPNs) we could find will be presented.

MATERIALS AND METHODS

Subjects

Peripheral blood leukocytes or lymphoblastoid cell lines from 30 cases of idiopathic MR associated with some dysmorphic features (14 males and 16 females) and their parents, when available, were sent to us after informed consent. MR patients, except for sisters, MR123 and MR124, were sporadic. One phenotypic female MR case, MR118 with showed obvious cytogenetic abnormality, 46,XY, but other MR cases had a normal karyotype according to G-banded chromosomal analysis at the 400-band level. Their metaphase chromosomes for FISH and DNA for aCGH were prepared according to standard protocols. We also used DNA from an individual with an abnormal karyotype [46,XX,inv dup del(8)(qter → p23.1::p23.1 → p11.23:)] for a positive control, and from a normal male and a female as sex-matched references.

Degenerate Oligonucleotide Primed PCR (DOP-PCR)

BAC/PAC DNA was amplified by two-step PCR. First DOP-PCR was performed using three different primers [Fiegler et al., 2003] as previously described [Harada et al., 2004]. The second 5'-amino-linked primer (5'-GGAAACAGCccgactcgag-3') whose 3' 10 base-pairs (small character) are the same as 5' 10 base-pairs of the first DOP-PCR primers. The second PCR was performed in a total volume of 100 μl containing 1 μl of the mixture of the three different first-PCR products, 1 × Ex Taq buffer, 5 U Ex Taq (Takara, Ohtsu, Japan), 0.2 mmol/L each dNTP, and 1 μmol/L second DOP-PCR primer, and the PCR was cycled 35 times at 94°C for 30 sec, 50°C for 30 sec, and 72°C for 8 min. These four primers were purchased from Hokkaido System Science Co. Ltd. (Sapporo, Japan).

Whole Genome Array

We selected 2,505 "FISHed" BAC/PAC clones using UCSC genome browser [2003 July version

(http://genome.ucsc.edu/cgi-bin/hgGateway)], spacing at every 1.4 Mb of the whole human genome and chose 2,173 clones as they showed a unique signal at the predicted chromosomal location. A total of 332 clones were not used for array study, as 172 yielded multiple chromosomal signals by FISH (6.8%) and 160 showed an aberrant signal that is probably due to contamination (6.4%). Fifty-nine BAC/PAC clones previously used for subtelomere and syndromic MR-specific microarray [Harada et al., 2004; Kurosawa et al., 2004] were also incorporated into 2,173 clones. BAC/PAC DNA was extracted using an automatic DNA extraction system PI-100 (Kurabo, Osaka, Japan), amplified by two-round PCR, purified and adjusted to the final concentration >500 ng/μl, and spotted in duplicate on CodeLink™ activated slides (Amersham Biosciences Corp, Piscataway, NJ) by the ink-jet spotting method (Nihon Gaishi, Nagoya, Japan). The identical set of duplicated spots was printed twice as block A and block B on one slide.

FISH

BAC/PAC DNA was labeled with Spectrum-Green™-11-dUTP or SpectrumOrange™-11-dUTP (Vysis, Downers Grove, IL) 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 hr, then washed and mounted in antifade solution (Vector, Burlingame, CA) containing DAPI. Fluorescence photomicroscopy was performed as previously described [Miyake et al., 2004].

Array CGH

After complete digestion using EcoRI, subject's DNA was labeled with Cy-3 dCTP (Amersham Biosciences) and reference DNA was labeled with Cy-5 dCTP (Amersham Biosciences) using DNA random primer Kit (Invitrogen, Carlsbad, CA) (CGH1). Dyes were swapped in CGH2 (subject DNA with Cy5 and reference DNA with Cy3) to check whether signal patterns of CGH1 were reversed for ruling out false positives. Prehybridization and hybridization were preformed as previously described [Harada et al., 2004]. Slides were incubated at 37°C for 72 hr with gentle shaking and were washed once with solution A (1 × PBS with 0.05% Tween 20), and twice with solution B (2 × SSC with 50% Formamide) at 43°C for 15 min, and twice with solution A at room temperature for 10 min with gently shaking. After drying, the arrays were scanned by GenePix 4000B (Axon Instruments, Union City, CA) and analyzed using GenePix Pro 4.0 (Axon Instruments).

The signal intensity ratio between patient's and control DNA was calculated from the data of the