出された 2q27.3 モノソミーの分子細胞遺伝学的解析．日本人類遺伝学会第 52 回大会

大村奈緒美，森内美由紀，佐々木健作，国場英雄，近藤達郎，松本直通，吉浦孝一郎，新川詔夫，原田直樹：t(1;3)(p13;q25) 相互転座切断点に微細欠失を認めた精神遅滞児の 1 例．日本人類遺伝学会第 52 回大会

国場英雄，霜川 修，Liang Desheng，Xia Jiahui，木下 晃，吉浦孝一郎，原田直樹，近藤達郎，大橋博文，黒澤健司，福嶋義光，成富研二，新川詔夫：歌舞伎メーキャップ症候群の染色体転座・微細欠失内の候補遺伝子解析．日本人類遺伝学会第 52 回大会

佐々木健作，霜川 修，川良洋城，国場英雄，近藤達郎，夫 律子，本多敬輔，松本直通，吉浦孝一郎，新川詔夫，原田直樹：未培養羊水の全ゲノム増幅による出生前診断の試み．日本人類遺伝学会第 52 回大会

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原田直樹，佐々木健作，霜川 修，川良洋城，冨士山龍伊，近藤達郎，夫律子，松本直通，吉浦孝一郎，新川詔夫：マイクロアレイを使用した全ゲノムコピー数解析による出生前診断の試み．第 32 回日本遺伝カウンセリング学会

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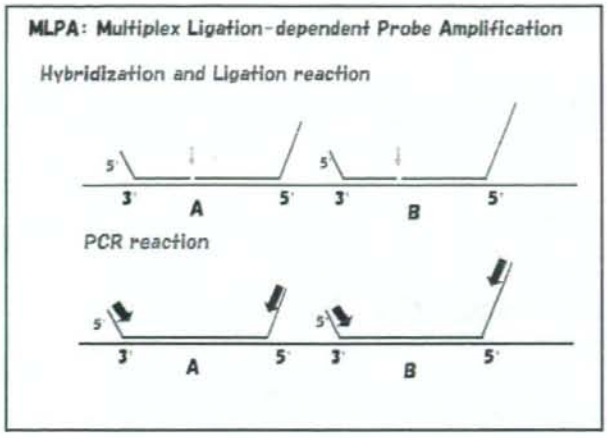
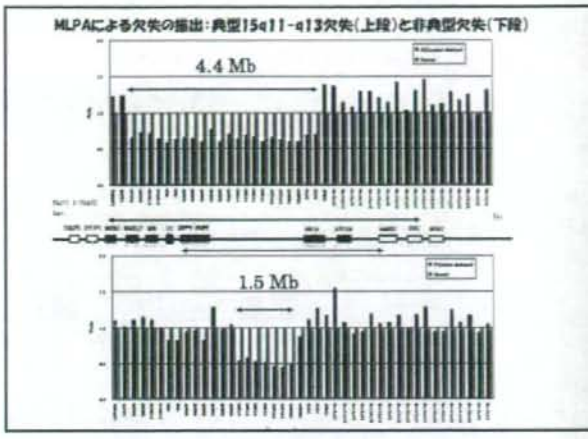
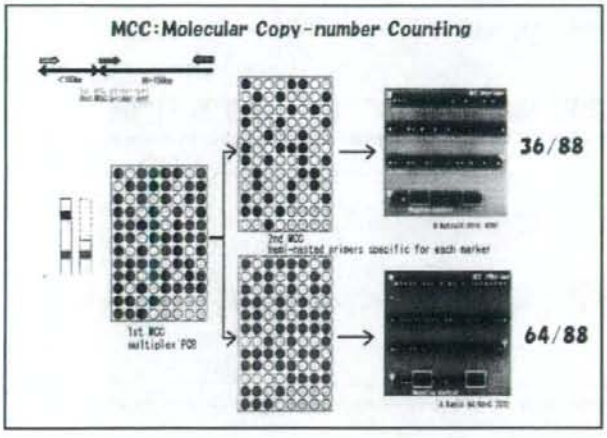
原田直樹：マイクロアレイを使用した全ゲノムコピー数解析による染色体検査の有用性と問題点．日本人類遺伝学会第 53 回大会

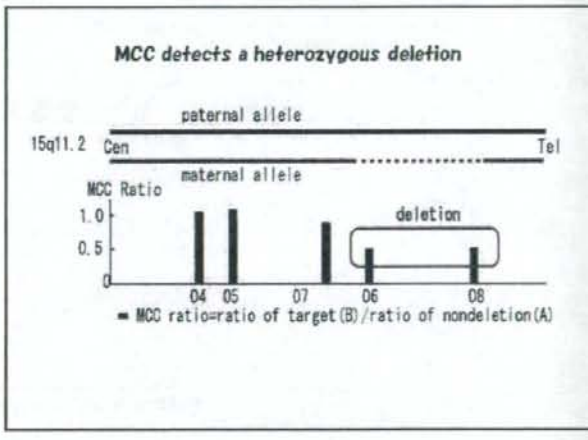
G．知的財産権の出願・登録状況
特記事項無し

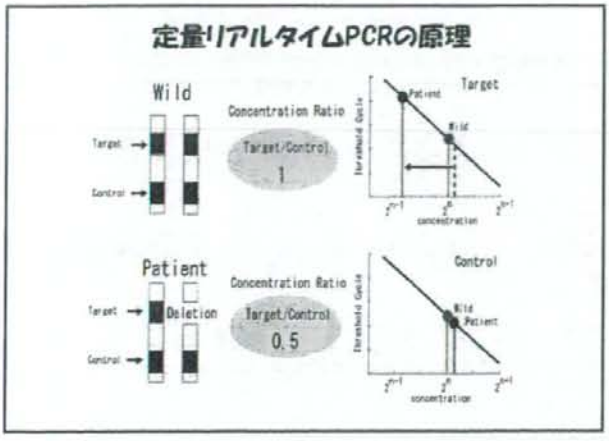
図１．アレー解析結果の各種検証法の検討











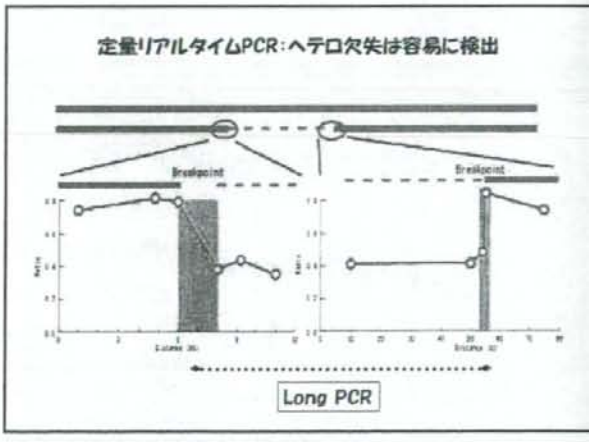


図２．各種キットを用いたWGA法とアレー解析結果

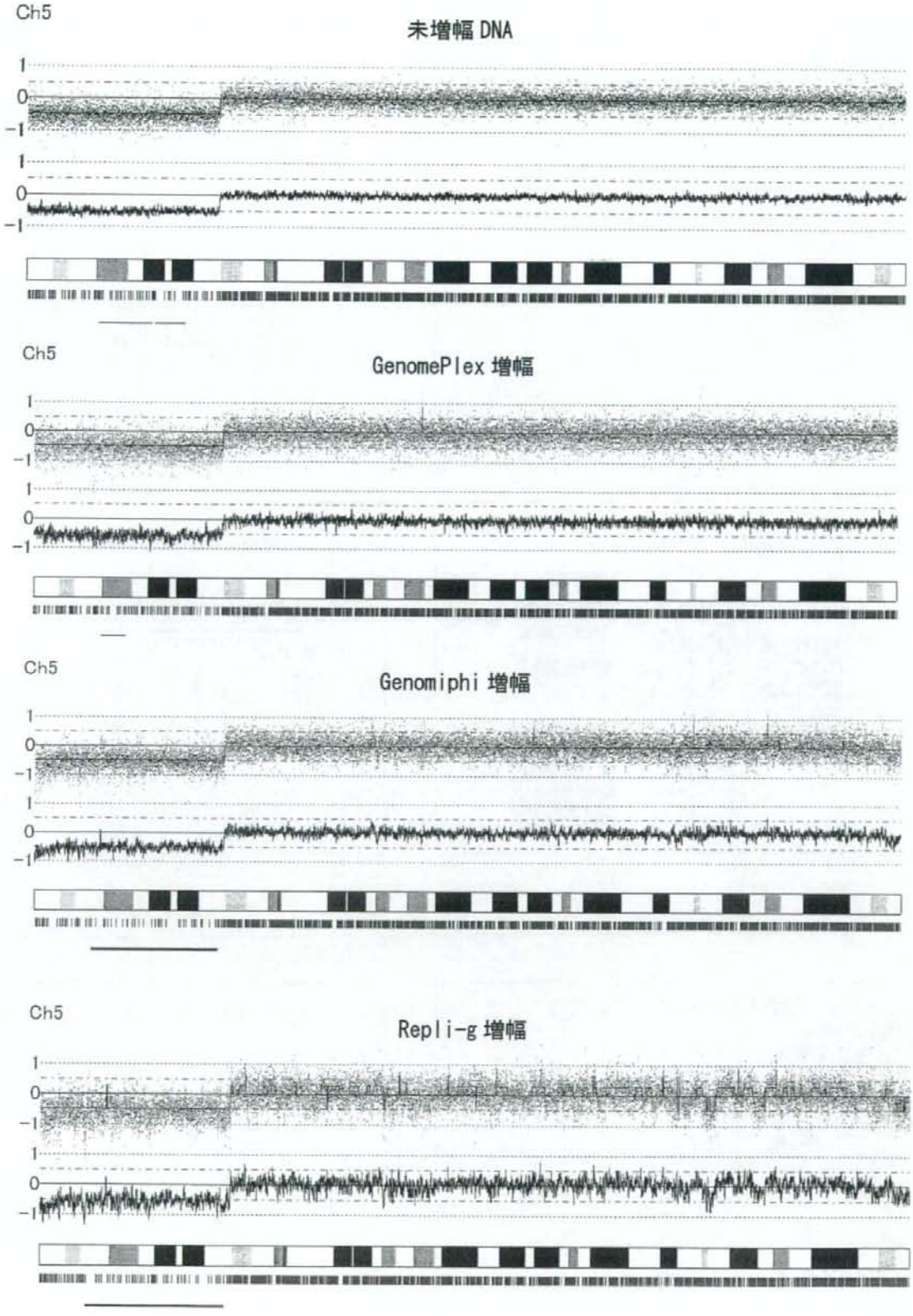


図3. 250K Nspアレーで検出したCNV

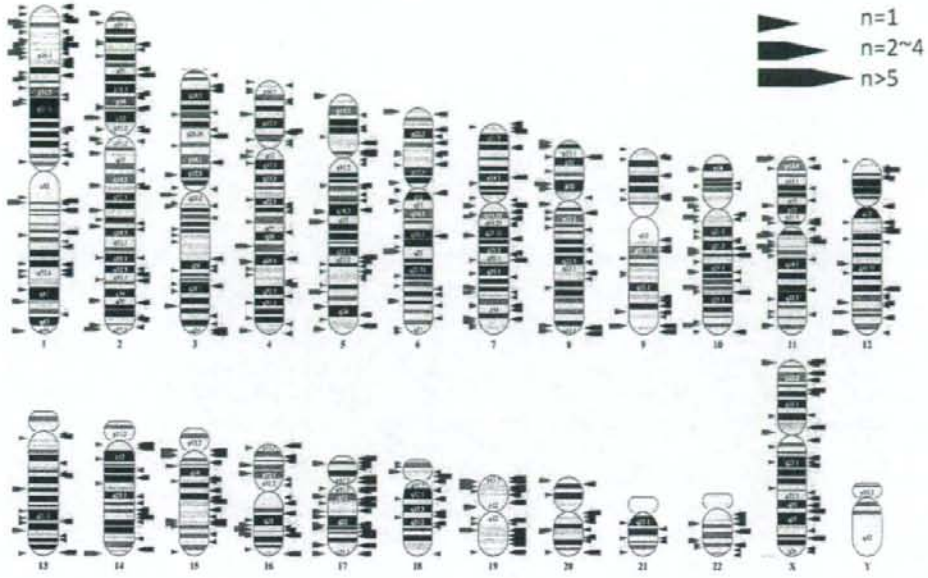


図4. SNP6.0アレーで検出したCNV

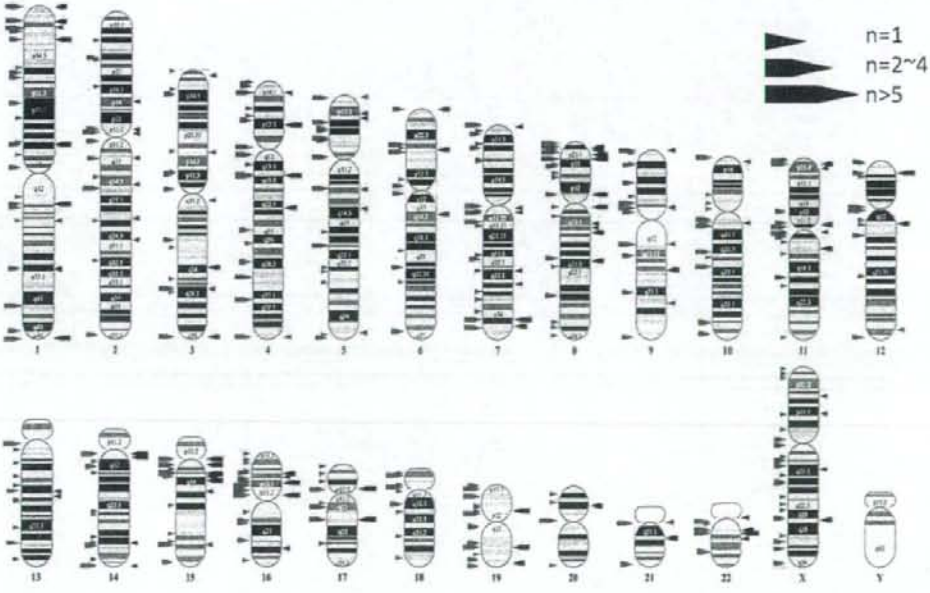


図3・4とも青は重複・赤は欠失、nは症例数を示す。

研究成果の刊行に関する一覧表

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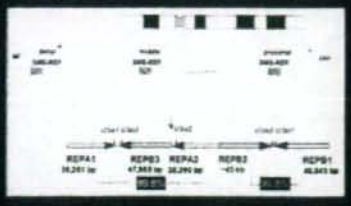

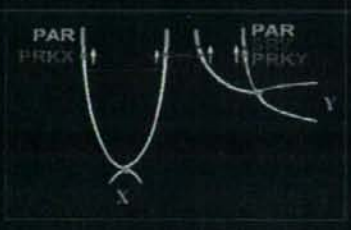

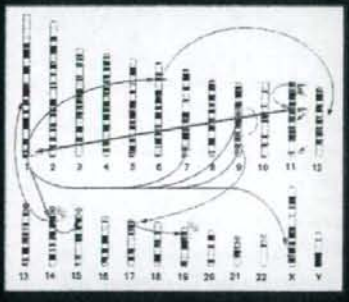

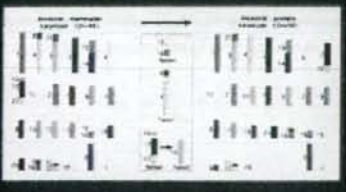

Genomic Disorders

The Genomic Basis of Disease

Edited by

James R. Lupski, MD, PhD

Paweł Stankiewicz, MD, PhD

HUMANA PRESS

16 Sotos Syndrome

Naohiro Kurotaki, MD, PhD and
Naomichi Matsumoto, MD, PhD

Contents

INTRODUCTION

Sotos syndrome (SoS) is a well-known overgrowth syndrome with mental retardation, specific craniofacial features, and advanced bone age. Since *NSD1* haploinsufficiency was proven to be the major cause of SoS in 2002, many intragenic mutations and chromosomal microdeletions (MDs) involving the entire *NSD1* gene have been described. The sizes of most SoS MDs are identical and a specific genomic architecture around these MDs was found. Recently, precise analyses of the low-copy repeats (LCRs) flanking the SoS common deletion showed that the deletion arises through nonhomologous recombination (NAHR) utilizing the LCRs, and proved that SoS is a genomic disorder.

SoS (OMIM no. 117550), also known as cerebral gigantism, was originally reported by Sotos et al. *(1)* in 1964. SoS is characterized by overgrowth, characteristic craniofacial features, developmental delay, and advanced bone age *(2)*. In 2002, *NSD1* disruption was found in a patient with SoS and haploinsufficiency of *NSD1* has been shown to be a major cause of SoS *(3)*. In the Japanese population, about half cases have chromosomal MDs involving the entire *NSD1* gene *(3,4)*. The majority of MDs are identical *(4)* and the NAHR was shown to be a causative mechanism *(5,6)*. In this chapter, we focus on recent progress in clinical and genetic aspects of SoS. The genomic architecture around the common MD will be presented.

From: *Genomic Disorders: The Genomic Basis of Disease*
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CLINICAL FEATURES

SoS typically presents with overgrowth, characteristic craniofacial features, developmental delay, and advanced bone age *(2)*.

Overgrowth: overgrowth usually starts prenatally and can be observed especially in early infancy *(2)*. However, overgrowth seems to normalize during childhood and may not be found in adulthood *(7–9)*.

Craniofacial features: macrocephaly, a high hairline with coarse hair growth, high arched palate, and prominent jaw are common findings. The occipitofrontal circumference is more than 97th percentile in childhood *(2)*.

Developmental delay: developmental delay is a cardinal symptom and can be attributed to central nervous system abnormalities *(2)*. Speech delay and abnormal motor development are commonly observed. Dilatation of cerebral ventricles is sometimes noted. In neuroimaging studies of 40 SoS patients, prominence of the trigone and the occipital horns was found in 90 and 75%, respectively *(10)*.

Advanced bone age: advanced bone age was observed in 31 of 37 (84%) SoS patients *(2)* and does not appear to be associated with abnormalities of collagen metabolism *(11)*.

Others: cardiac, urogenital, musculoskeletal, and ophthalmologic anomalies have been observed also *(2,12–15)*. Neoplasms in SoS have been found with a frequency of 2.2–3.9% *(16)*.

DIAGNOSIS OF SOS

SoS has been diagnosed based on clinical manifestations, however, the diagnosis is difficult when craniofacial features are less remarkable or are similar to those of other overgrowth syndromes with overlapping phenotypes. Weaver and Beckwith-Wiedemann syndromes associated with *NSD1* abnormalities showed similar facial features to those of SoS *(17,18)*. Overgrowth tends to normalize in adulthood *(9)*, thus, the diagnosis is easier in early life *(19)*. Objective diagnosis of SoS has been substantially improved, since the discovery of *NSD1* mutations in SoS *(3)*. The major diagnostic criteria proposed by Cole and Hughes *(2)* include pre- and postnatal characteristic overgrowth with advanced bone age, and developmental delay. However, a recent report by Rio et al. *(20)* indicated that typical facial appearance and macrocephaly were consistently recognized but overgrowth or advanced bone age was not always observed in patients with *NSD1* mutations. Therefore, the diagnostic criteria for SoS likely need to be carefully revised after collecting more data on the phenotypic spectrum in SoS patients with known *NSD1* abnormalities. "Sotos-like syndrome" usually has been referred to a large spectrum of patients that do not fulfill the major criteria for SoS *(2,17,20,21)*.

MOLECULAR GENETICS

NSD1 Structure and Functions

Mouse *Nsd1* was originally isolated in 1998 as one of the nuclear proteins interacting with retinoic acid receptors and thyearsoid hormone receptors *(22)*. The human *NSD1* was identified at the 5q35 breakpoint of a SoS patient with an apparently balanced translocation t(5;8)(q35;q24.1) *(23,24)*. *NSD1* has an 8088-bp open reading frame consisting of 23 exons and is translated into a 2696 amino-acid protein. NSD1 protein has at least six functional domains, a su[var]3-9,enhancer-of-zest,trithorax (SET) domain, two proline-tryptophan-tryptophan-proline (PWWP) domains, and three plant homeodomain protein-finger (PHD)

domains with nuclear localization signals. The SET domain of NSD1 showed methyl transferase activity for Lys36 of histone H3 (H3-K36) and Lys20 of histone H4 (H4-K20), whose methylation may result in transcriptional silencing of developmentally regulated genes *(25)*. PHD finger domains are found in a large number of chromatin regulatory factors like CBP/p300, and chromatin remodeling protein ACF *(26)*. The PWWP domain was initially identified in the protein encoded by the Wolf-Hirschhorn syndrome candidate gene 1 (*WHSC1*) *(27)*. The essential function is still unknown, however, the PWWP domain is often associated with SET domains and is thought to be an essential for development *(28)*. Thus, these six protein domains are suggested to regulate chromatin formations and gene transcriptions *(23)*. Homozygous knockout of *Nsd1* in mice led to embryonic death at 10.5 days and gastrulation failure, suggesting that Nsd1 may be a protein regulating development especially in an early post-implantation period *(25)*.

The NSD1 is a portion of a fusion protein in a recurrent translocation t(5;11)(q35;p15.5) found in childhood acute myeloid leukemia *(29–31)*. Six non-SoS cases of acute myeloid leukemia had fusion transcripts of *NSD1* and *NUP98* (nucleoporin 98 gene) *(29–31)*. SoS has been suggested to be associated with neoplasms *(32)*. It is also important to address whether *NSD1* abnormality in itself can cause tumorigenesis in the near future.

NSD1 Mutations

NSD1 mutations in SoS have been reported by several groups *(3,4,17,20,33–36)*. To date, among a total of 91 point mutations (PM) have been identified in 241 patients suspected of SoS. Seventy-one protein truncation mutations and 20 missense mutations have been reported with 62 being *de novo* (Fig. 1). Five protein truncation mutations were found in 23 Sotos-like patients *(17,20)*. Protein truncation mutations are spread throughout the entire *NSD1* coding regions; however, missense mutations cluster at the 3' part of *NSD1* where most of the known functional domains are located. In SoS, six missense mutations have been identified in the SET domain, three in the PHD domains and three in the PWWP domains.

Thus, among different world populations, *NSD1* mutations have been consistently shown to be the major cause of SoS. Interestingly, the frequency of MDs involving *NSD1* is quite different. In the Japanese population, about half of the cases (49/95) had MDs, whereas microdeletions are observed in only 11% of cases (13/118) analyzed in European populations *(3,4,17,20,33–36)*. The reason for this observed difference remains to be elucidated.

Genotype/Phenotype Correlation

Interestingly, some clinical differences between SoS patients with PMs and MDs have been reported *(14,17,20)*. Nagai et al. *(14)* compared clinical phenotypes between 5 PM and 21 MD SoS patients. Both PM and MD cases showed typical craniofacial features. Remarkably, the peak height at younger than 6 years of age and the intelligence quotient/developmental quotient (IQ/DQ) in patients older than 6 years were significantly different between PM and MD patients. The values of the standard deviation (SD) scores were 3.3 (PM) and 2.2 (MD), and IQ/DQ (mean) were 78 ± 12 (PM) and 57 ± 12 (MD), respectively. In addition, MD patients predominantly showed cardiovascular and urogenital abnormalities, and recurrent convulsions.

Rio et al. *(20)* compared 16 PM and 6 MD patients. Two MD patients showed typical SoS, but the other four patients were diagnosed as Sotos-like syndrome because they did not have overgrowth (height less than +2 SDs), or advanced bone age. Four out of six MD patients had severe mental retardation with no speech at all. Cardiovascular anomalies were found in three

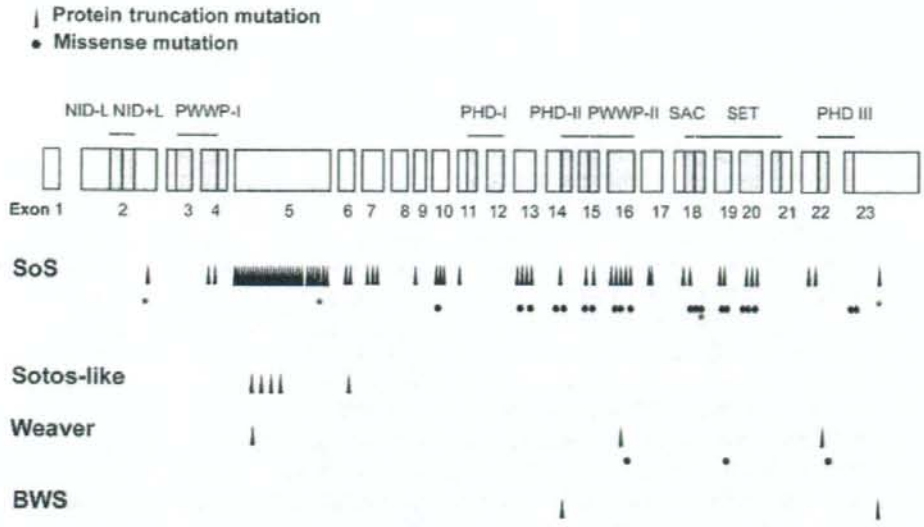

Fig. 1. The structure of *NSD1* with mutations in Sotos syndrome, Sotos-like syndrome, and other syndromes. Each box indicates exon, gray regions show functional domains (SET, PWWP, and PHD) and nuclear receptor interacting domains, NID^{-L} and NID^{+L}. Start and stop codons are located at exon 2 and 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 cardiovascular 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,

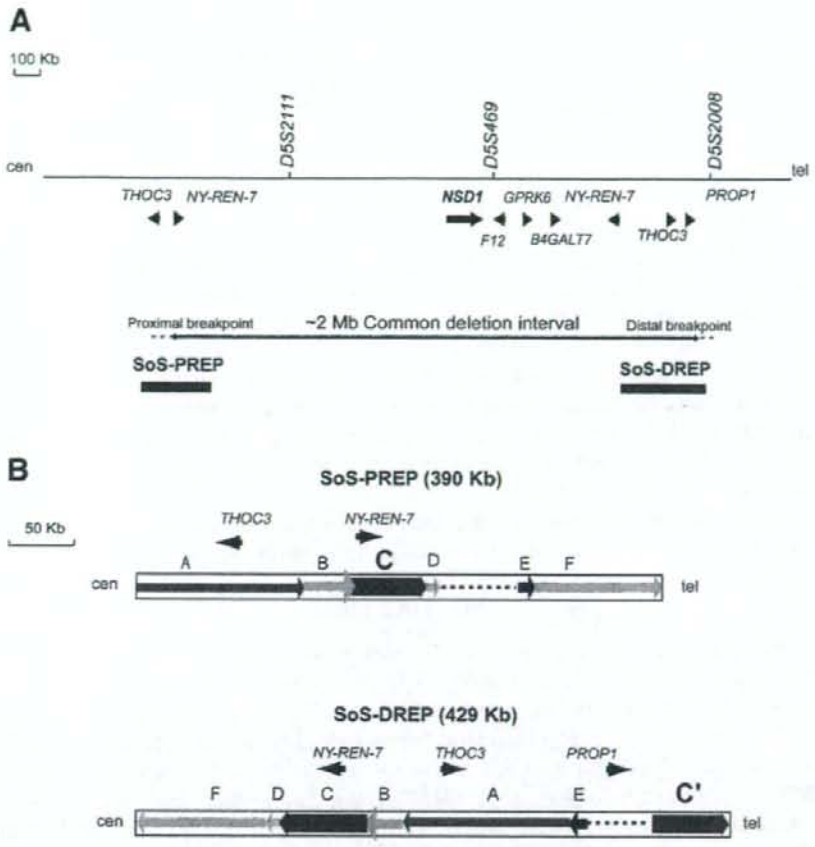

Fig. 2. (A) Physical map depicting microdeletions found in Sotos syndrome (SoS) and two low-copy repeat sequences, termed proximal-repeat (SoS-PREP) and distal-repeat (SoS-DREP) at 5q35. The Sos-REPs, indicated as black boxes, are proximal and distal to *NSD1*. Among 22 genes that map within the deletion 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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