of two types of microarray, BAC array and oligonucleotide array. The BAC array was applied for 298 patients to detect 58 CNVs in 47 patients, and among them 26 CNVs (8.7%) were determined to be causal (pathogenic). Conversely, the oligonucleotide arrays were applied for 703 patients to detect 1538 CNVs in 603 patients, and among them 74 CNVs (10.5%) were determined to be pathogenic. These results may lead to the following idea: a lower-resolution microarray detects a limited number of CNVs likely to be pathogenic, because such CNVs tend to be large, and a higher-resolution microarray detects an increasing number of bCNVs or VOUS.[38] Indeed, in studies using a high-resolution microarray, most of the CNVs detected were smaller than 500 kb but almost all pCNVs were relatively large.[54,81,83] Most of the small CNVs were judged not to be pathogenic, and the percentage of pCNVs stabilized at around 10%. This percentage may suggest a frequency of patients with MCA/MR caused by CNV affecting one or more genes, other than known syndromes and subtelomeric aberrations. The other patients may be affected by another cause undetectable by genomic microarray; for example a point mutation or microdeletion/duplication of a single gene, aberration of microRNA, aberration of methylation states, epigenetic aberration or partial uniparental disomy.

As recently hypothesized secondary insult, which is potentially another CNV, a mutation in a phenotypically related gene or an environmental event influencing the phenotype, may result in clinical manifestation.[84] Especially, in two-hit CNVs, two models have been hypothesized: (1) the additive model of two co-occurring CNVs affecting independent functional modules and (2) the epistatic model of two CNVs affecting the same functional module.[85] It also suggests difficulty in selecting an optimal platform in the clinical screening. Nevertheless, information on both pCNVs and bCNVs detected through studies using several types of microarrays is unambiguously significant because an accumulation of the CNVs will create a map of genotype–phenotype correlation that would determine the clinical significance of each CNV, illuminate gene function or establish a new syndrome.

ACKNOWLEDGEMENTS

We thank Ayako Takahashi and Rumi Mori for technical assistance. This study was supported by the Joint Usage/Research Program of Medical Research Institute, Tokyo Medical and Dental University. This work was also supported by grants-in-aid for Scientific Research on Priority Areas from the Ministry of Education, Culture, Sports, Science, and Technology, Japan; a grant from Core Research for Evolutional Science and Technology (CREST) of the Japan Science and Technology Corporation (JST); a grant from the New Energy and Industrial Technology Development Organization (NEDO); and in part by Grant-in-Aid for Scientific Research (B) (17390099, 20390301) of Japan Society for the Promotion of Science (JSPS); Health and Labour Sciences Research Grants for Research on information system of undiagnosed diseases (H21-nanchi-ippan-167) and Research on policy for intractable diseases (H22-nanchi-shitei-001) from the Ministry of Health, Labour and Welfare, Japan.

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CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

Clinical and Genomic Characterization of Siblings With a Distal Duplication of Chromosome 9q (9q34.1-qter)

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Received 21 July 2010; Accepted 23 May 2011

We report herein on two female siblings exhibiting mild intellectual disability, hypotonia in infancy, postnatal growth retardation, characteristic appearance of the face, fingers, and toes. Their healthy mother had a translocation between 9q34.1 and the 13pter. FISH and array CGH analysis demonstrated that the two children had an additional 8.5 Mb segment of the 9q34.1-qter at 13pter. The clinical features of the present cases were similar to those of previously reported 9q34 duplication cases; however, the present cases did not exhibit other abnormal behaviors, such as autistic features or attention deficit disorders, those are reportedly associated with 9q34 duplications. A 3.0 Mb region (9q34.1-q34.3) within 9q34 duplication in our patients are overlapped with duplication region of previously reported cases and is proposed to be critical for the presentation of several phenotypes associated with 9q34 duplications.

Key words: 9q34 duplication; intellectual disability; array CGH; dysmorphism

How to Cite this Article:
Mizuno S, Fukushi D, Kimura R, Yamada K, Yamada Y, Kumagai T, Wakamatsu N. 2011. Clinical and genomic characterization of siblings with a distal duplication of chromosome 9q (9q34.1-qter).
Am J Med Genet Part A 9999:1–7.

INTRODUCTION

Duplications of a distal region of the long arm of chromosome 9 (9q34) are rare and few cases have been reported. The first association between 9q34 duplications and phenotypic abnormalities were demonstrated in seven cases in a large pedigree [Allderdice et al., 1983]. The patients had low birth weight, initial poor feeding and thriving, slight psychomotor retardation, characteristic appearance of the face, fingers, and toes. Hyperactive behavior, heart murmur, and ptosis and strabismus were also noted. In another case, a girl of 3 years and 2 months carried a 9q34 duplication and a deletion of 3p26-pter due to a balanced translocation in her mother [Hodou et al., 1987]. This patient presented with dolichocephaly, characteristic facial appearance, and long thin fingers and toes, all of which are phenotypes noted in previous cases of 9q34 duplication; she also exhibited features associated with 3p terminal monosomy. In addition, duplication of 9q34-qter and monosomy of a small region on 12p13.3 in a male infant was described by Spinner et al. [1993]. The same patient was followed up at 18 years of age, and the duplicated and deleted regions were determined in detail by array-based comparative genomic hybridization (array CGH) analysis [Youngs et al., 2010]. The patient exhibited autistic features, hyperactivity, and attention deficit disorder in addition to the features associated with 9q34 duplications reported previously. Gawlik-Kuklinska et al. [2007] reported the case of a 17-year-old girl with an interstitial 7.4 Mb duplication of 9q34.1-q34.3 determined by array CGH analysis and compared the clinical features of the patient with those of previous cases. This patient exhibited the features common to patients with 9q34 duplications and three additional phenotypes of food-seeking behavior, obesity, and secondary amenorrhea.

In this report, we present two female siblings with 9q34.1-qter duplications and compare the clinical features and 9q34 duplication region of these patients with those of two previously reported cases using array CGH analysis. We also discuss the loci potentially responsible for the several phenotypes associated with a specific segment of 9q34.

Additional supporting information may be found in the online version of this article.

Grant sponsor: Takeda Science Foundation; Grant sponsor: Health Labour Sciences Research Grant.

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Published online 8 August 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.34160

CLINICAL REPORTS

Patient 1. The patient was a 4-year-old girl and the first child of healthy, non-consanguineous Japanese parents. The family history was unremarkable. She was born at 40 weeks of gestation weighing 2,564 g and measuring 47.3 cm in length with an occipitofrontal circumference (OFC) of 33 cm, all within the standard range (10th–90th centile) for female Japanese neonates. The child was first evaluated at a cardiology clinic to investigate a heart murmur in the neonatal period. She was diagnosed with Ebstein anomaly, which was surgically repaired when she was 2-month old. At the age of 4 months, she was referred to our hospital due to generalized hypotonia and developmental delay. She rolled over at 12 months and sat up at 18 months. She stood with support at 24 months and started to walk unaided at 2.5 years. At 3 years of age, her height was 84 cm (−2.2 SD), body weight was 12.4 kg (−0.7 SD), and OFC was 49 cm (−0.2 SD). She could speak several meaningful words and understand simple sentences. Her developmental quotient (DQ) was 67, indicating mild intellectual disability. She was a sociable and friendly girl.

Clinical examination revealed that she had a characteristic facial appearance, including a round face, hypertelorism, almond-shaped palpebral fissures, telecanthus, depressed nasal bridge, short nose, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip (Fig. 1A). Her fingers were slender but not tapered (Fig. 1C). Neurological examination revealed that the cranial nerves were intact except for strabismus. Ocular fundi were normal. She walked slowly, but no ataxia was evident. Muscle tonus of the extremities was normal. Tendon reflexes of extremities were normal, and pathological reflex was absent. There was no evidence of epilepsy. Routine laboratory investigations were normal.

Patient 2. The patient was a 3-year-old girl and was the second child of the parents of Patient 1. She was born at 40 weeks of gestation weighing 2,874 g, measuring 49 cm in length with an OFC of 34.3 cm (all normal values for female Japanese neonates). She exhibited generalized hypotonia, but no feeding problems were observed during the neonatal period. She was referred to our hospital at the age of 19 months due to developmental delay. She exhibited head control at the age of 4 months. She rolled over at 9 months, sat at 10 months, and cruised between 11 and 12 months. She started to walk unaided at 18 months. Her height at 3 years was 88 cm (−2.4 SD), body weight was 10.1 kg (−2.7 SD), and OFC was 47 cm (−0.7 SD). DQ at the age of 3 was 72, indicating mild intellectual disability. She routinely exhibited affectionate and sociable behavior. She also had a round face with full cheeks, hypertelorism, almond-shaped palpebral fissures, telecanthus, depressed nasal bridge, short nose, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip (Fig. 1B). Ultrasonography of the abdomen showed no urogenital defects. No ophthalmic anomalies other than strabismus were found on routine evaluation. Neurological examination was not remarkable except strabismus. No epileptic seizures were observed. Routine laboratory investigations were normal. The clinical features of both patients and two previously reported cases of 9q34 duplication are summarized in Table I.

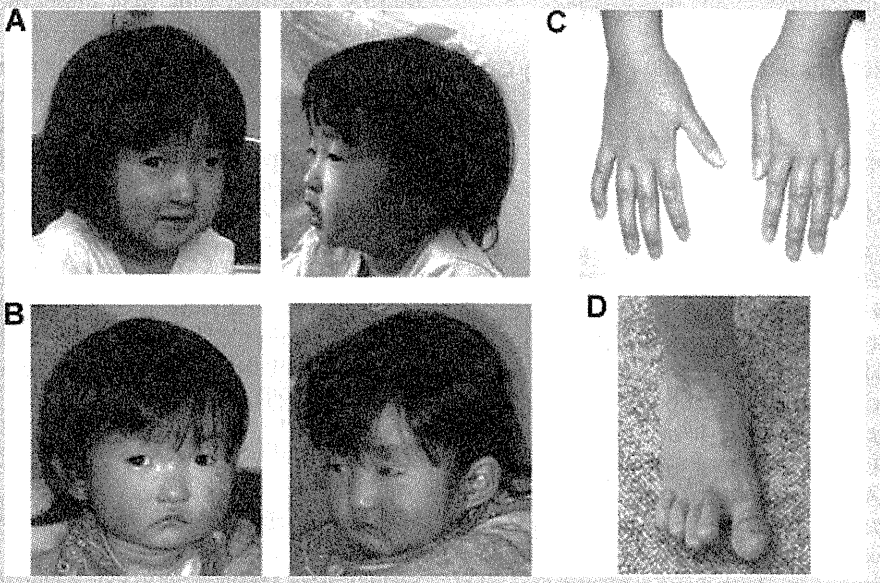

FIG. 1. A: Frontal and lateral views of Patient 1 at 3 years of age. Phenotypes include round face, hypertelorism, telecanthus, short nose, depressed nasal bridge, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip. B: Frontal and oblique view of Patient 2 at 2 years of age. Phenotypes include round face, hypertelorism, almond-shaped palpebral fissures with telecanthus, short nose, depressed nasal bridge, microstomia, microretrognathia, short philtrum, and Cupid's bow upper lip. C: Hands of Patient 1 with long and thin fingers. D: The right foot of Patient 1. She has long toes with increased space between the first and second toes.

TABLE I. Clinical Features of Patients With a 9q34.1-qter Duplication

Phenotypic features	Gawlik-Kuklinska et al. [2007]	Youngs et al. [2010]	Patient 1	Patient 2
General				
Hypotonia	+	+	+	+
Failure to thrive	+	−	−	−
Intellectual disability	Mild	Mild	Mild	Mild
Cardiac anomalies	−	+	+	−
Overweight/obesity	+	+	−	−
Scoliosis	+	−	−	−
Facial characteristics				
Dolichcephaly	+	+	−	−
Facial asymmetry	+	+	−	−
Narrow horizontal palpebral fissures	+	+	−	−
Deep-set eyes	+	+	−	−
Long nose	+	+	−	−
Prominent chin	+	+	−	−
Microstomia	+	+	+	+
Microretrognathia	+	+	+	+
Short philtrum	+	−	+	+
Round face	−	−	+	+
Hypertelorism	−	−	+	+
Depressed nasal bridge	−	−	+	+
Almond-shape palpebral fissures	−	−	+	+
Telecanthus	−	−	+	+
Short nose	−	−	+	+
Extremities				
Long and thin fingers	+	+	+	+
Increased space between first and second toes	+	+	+	+

+, present; −, absent.

MATERIALS AND METHODS

Cytogenetic Analysis

Cultured lymphoblastoid cells isolated from each patient were treated with colchicine (Sigma–Aldrich, St. Louis, MO) for 1 hr at a concentration of 20 ng/ml in culture medium, and then incubated in a hypotonic solution of 75 mM KCl at 37°C for 30 min. After incubation, cells were fixed with Carnoy's fixative (3:1 mixture of methanol and acetic acid), spread on glass slides in a humid atmosphere and air-dried. Chromosomal analysis was carried out on GTG banded chromosomes at a resolution of 400–550 bands. Fluorescence in situ hybridization (FISH) was performed on metaphase chromosome spreads from each patient. Commercial probes covering subtelomeric regions were used according to the manufacturer's protocols (ToTelVysion, Abbott Laboratories. Abbott Park, IL) [Flint et al., 1995]. In order to confirm the chromosomal rearrangement in detail, additional FISH analysis was carried out from the patients and their parents using a series of bacterial artificial chromosome (BAC) clones (Clontech Laboratories, Inc., Mountain View, CA) that map to chromosome regions 9q34 and 13q31.

Array CGH Analysis

Genomic DNA was isolated from peripheral blood lymphocytes of the two patients, their parents, and three normal controls by phenol/chloroform extraction. Array CGH analysis was performed using the Agilent Human Genome CGH 244K microarray platform (Agilent Technologies, Santa Clara, CA) according to standard protocols provided by the manufacturer. This array spans the entire human genome at a median resolution of approximately 8.9 kb. Genomic copy numbers were analyzed with Genomic Workbench (Standard Edition 5.0.14; Agilent Technologies).

Southern Blot Analysis

Genomic DNA samples (10 μg) from the patients, their parents, and the normal controls were digested with *Hin*dIII, separated on a 0.9% agarose gel, and transferred by the alkaline method to a nylon membrane (Hybond-N+; GE Healthcare, Tokyo, Japan). The membrane was sequentially hybridized with [α-^{32}P]dCTP-labeled *ABCA6* (exons 17–19) and *SP2* (exons 4–7) cDNA. A 301 bp *ABCA6* or a 798 bp *SP2* cDNA probe was prepared by amplifying the cDNA library of human lymphoblastoid cells with AmpliTaq-

Gold (Applied Biosystems, Foster City, CA) using specific primer pairs for *ABCA6* (sense: 5′-ATCTTTTCAGTGATCTGGATAAG-3′; antisense: 5′-AGGGTCAATAACACTTTAGTTT-3′), and for *SP2* (sense: 5′-GTCTACATCCGCACGCCTTC-3′; antisense: 5′-CCGCCGCAGTTGGCCTTA-3′), respectively. The PCR products were subcloned into pGEM-T easy vector (Promega, Madison, WI), and the nucleotide sequence of the probes was confirmed. Hybridization was performed in hybridization solution containing 5× standard saline citrate (SSC), 5× Denhardt's solution, and 0.5% SDS at 66°C overnight. The membrane was washed three times with 2× SSC containing 0.1% SDS at 37°C for 20 min and once with 0.1× SSC containing 0.1% SDS at 55°C for 10 min, and then radioactivity was quantified with a BAS 1800 image analyzer (FUJIFILM, Tokyo, Japan). The radioactivity of *ABCA6* versus *SP2* was determined for both patients and their parents (RP1, RP2, RF, RM) relative to the mean of the three normal controls (RC).

RESULTS

Additional 9q Subtelomeric Signal

The G-banding pattern of the both patients showed a 46,XX normal female karyotype. FISH with probes for subtelomeric regions revealed an additional 9q subtelomeric signal on the short arm of a D-group chromosome (chromosome 13, 14, or 15) in both patients (data not shown).

9q34 Duplication

To assess the chromosomal rearrangements in more detail, FISH analysis was performed in both patients and their parents with three BAC clones (RP11-40A7 and RP11-81N19) from chromosome 9q34 and RP11-524C15 from chromosome 13q31. The result indicated that the mother had a translocation; a 9q34.1-qter segment from one chromosome 9 was translocated to the terminus of chromosome 13p (Fig. 2, lower panel, indicated by a yellow arrow). Both patients had two normal chromosomes 9 and the derivative chromosome 13, which had an additional 9q34.1-qter segment at the p-terminal (Fig. 2, lower panels, indicated by yellow arrows). The father did not show any abnormalities (data not shown). These results indicate that the additional 9q34.1-qter segment at the p-terminal of chromosome 13 was of maternal origin (Fig. 2). The breakpoint of the translocation fell between two BAC clones at RP11-81N19 (129.2 Mb from the 9p terminus) and RP11-40A7 (133.4 Mb). Detailed mapping of the 13p breakpoint is not necessary because 13p does not code any genes. Thus, the duplicated segment was estimated to be 6.8–11.0 Mb derived from the 9q-terminus at position 140.2 Mb [46,XX.ish der(13)t(9;13)-(q34.1;pter)mat] (Fig. 2).

8.5 Mb Duplication of 9q34.1-qter

We performed array CGH using genomic DNA from each patient to determine the precise size of the additional 9q34 segment and

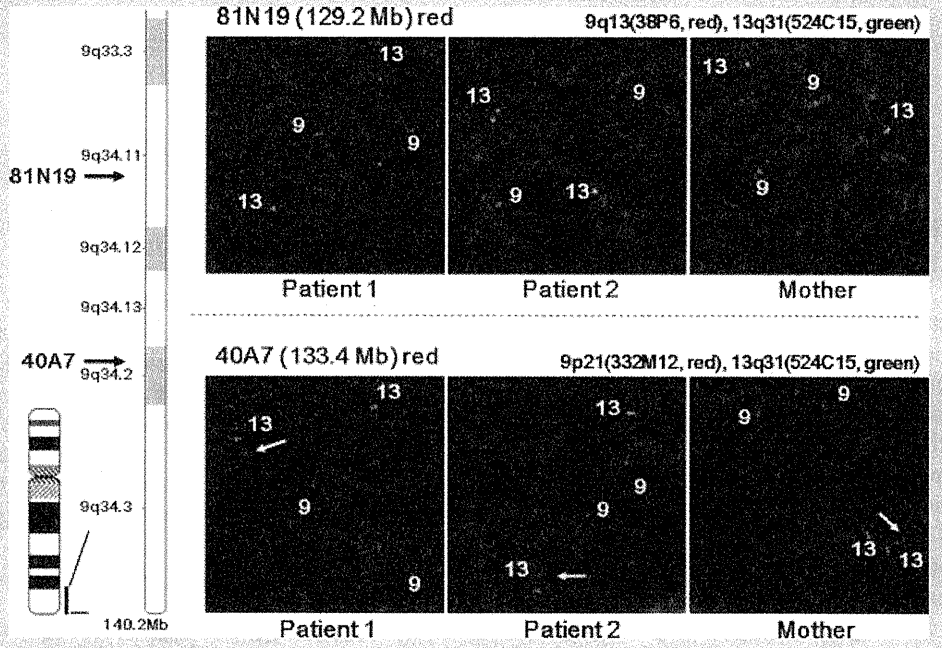

FIG. 2. Partial metaphases of FISH with BAC clone RP11-81N19 probe show two red signals on both 9q terminal regions of the mother and each patient (upper panel) and no signal on chromosome 13. Partial metaphases of FISH with BAC clone RP11-40A7 probe show a red signal on one 9q terminal region and the short arm of derivative chromosome 13 (yellow arrow) in the mother and three signals in both patients; two red signals on both 9q terminal regions and an additional signal on the short arm of derivative chromosome 13 (yellow arrow) (lower panel). RP11-38P6 (red), RP11-332M12 (red), and RP11-524C15 (green) are used as markers for 9q13, 9p21, and 13q31, respectively.

identify any other genomic abnormalities. Array CGH analysis of samples from Patients 1 and 2 demonstrated that the genomic copy number of 9q34.1-qter was 1.5-fold higher than the normal region (Fig. 3A,B). The size of the 9q34.1-qter duplication in both patients was approximately 8.5 Mb, from positions 131.7 to 140.2 Mb of chromosome 9 (Fig. 3). The breakpoint (position 131.7 Mb) of the 9q34 duplication in both patients was located in *FNBP1*, which encodes formin-binding protein 1. Analyses of Patients 1 and 2 revealed 12 and 15 copy number variations (CNVs), respectively (data not shown). CNVs are generally defined as the copy number differences of genomic DNA larger than 1 kb that vary in copy number between individuals. Patients 1 and 2 both had a 0.5-fold decrease in the genomic copy number of *ABCA6*, which encodes ATP-binding cassette, sub-family A, member 6; this is not recognized as a CNV (MIM 612504; Supplemental Fig. A and B).

ABCA6 Deletion in Both Patients and Their Mother

To confirm whether *ABCA6* was deleted in both patients and their parents, we performed Southern blot analysis using two cDNA probes against *ABCA6* (exons 17–19) and *SP2* (exons 4–7). *SP2* maps to 17q21, approximately 21 Mb proximal to *ABCA6*, and was not deleted in either patient based on the array CGH analysis. Southern blot analysis showed a decreased radioactive signal from *ABCA6* in family members (Supplemental Fig. C). When the mean ratio of *ABCA6* signal to *SP2* signal of the three normal controls was defined as 1.0, the ratio of *ABCA6* signal to *SP2* signal of the patients and their mother was approximately 0.5 and their father was 0.85 (Supplemental Fig. D). Thus, the both patients and their mother were heterozygous for an *ABCA6* deletion.

DISCUSSION

Duplications of 9q34 cause intellectual disability and multiple congenital anomalies. Reported cases presented with a variety of clinical features depending on the size of the duplication and the presence of other chromosomal abnormalities [Allderdice et al., 1983; Hodou et al., 1987; Spinner et al., 1993; Gawlik-Kuklinska et al., 2007; Youngs et al., 2010]. Our patients had a 9q34.1-qter duplication and partial 13p monosomy due to a translocation between 9q34.1 and 13pter in their healthy mother. Array CGH and Southern blot analyses confirmed that these patients had a 9q34.1-qter duplication and a heterozygous deletion of *ABCA6* (17q24). Because 13p does not code for any genes and the heterozygous deletion of *ABCA6* did not cause any phenotypic abnormalities in the mother, the present patients exhibited "pure" 9q34.1-qter duplications without any other chromosomal abnormalities involving coding genes.

9q34 duplication has been analyzed in detail using array CGH in only two other patients. Gawlik-Kuklinska et al. [2007] reported the case of the female with a 7.4 Mb (RP11-269P11 to RP11-295G24; 127.3–134.7 Mb) duplication of 9q34.1-q34.3 (Fig. 4) and compared the patient's clinical features to those of previously reported 9q34 duplication cases [Spinner et al., 1993], including a male patient later shown to have a 13.8 Mb (126.4–140.2 Mb) duplication of 9q33.3-qter [Youngs et al., 2010] (Fig. 4). The following

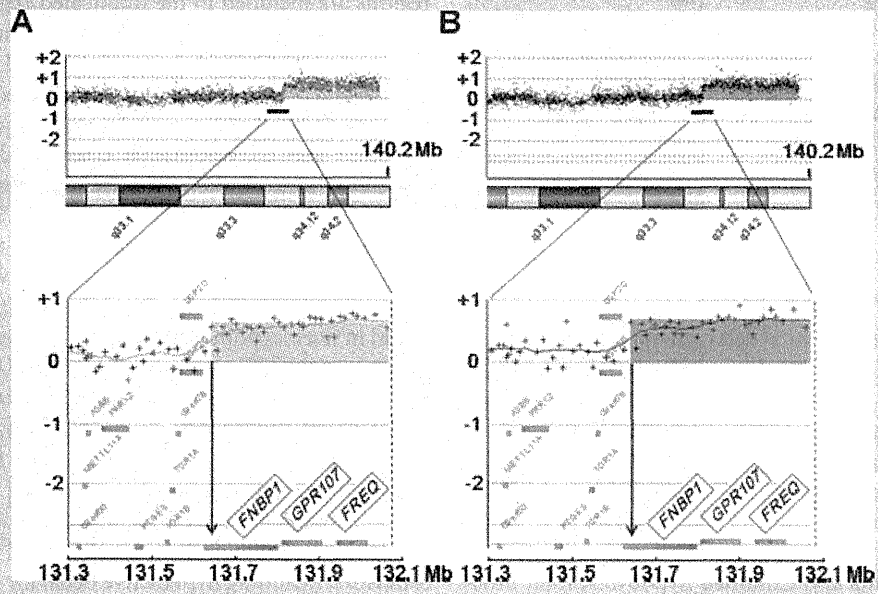

FIG. 3. A: Graphical representation of the results of the array CGH analysis (Agilent 244K oligonucleotide array) from Patient 1 shows the duplication of distal 9q34.1-qter (upper panel). The x- and y-axis denote genomic position and $\log_2$ ratio, respectively. B: Graphical representation of the results of the array CGH analysis from Patient 2 also shows the duplication of distal 9q34.1-qter (upper panel). The breakpoint in 9q34 was located in the *FNBP1* gene (131.7 Mb) in both patients (lower panels of A and B), which indicated that the size of the duplication was approximately 8.5 Mb (131.7–140.2 Mb) according to NCBI human genome build 36.3.

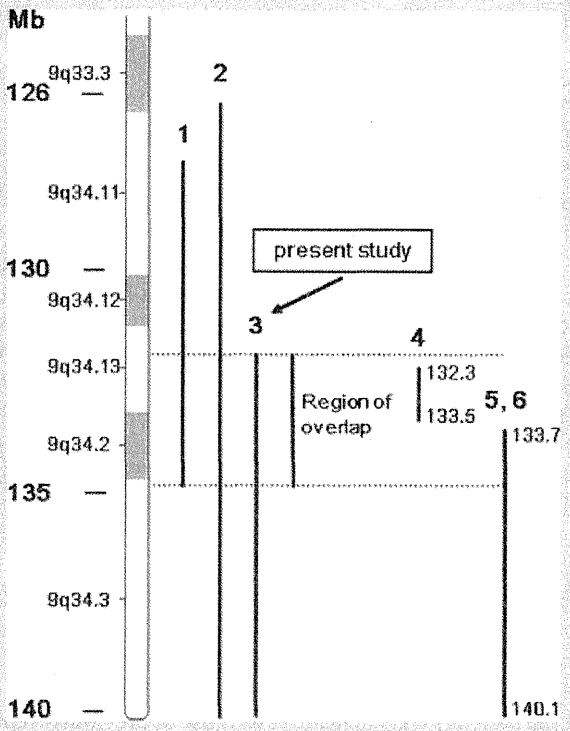

FIG. 4. A schematic illustration based on NCBI human genome build 36.3 of 9q34 duplications from two previously reported cases, the present patients, and three patients from DECIPHER. The duplications in the previously reported patients and our patients are denoted as 1 [127.3–134.7 Mb] [Gawlik-Kuklinska et al., 2007], 2 [126.4–140.2 Mb] [Youngs et al., 2010], and 3 [131.7–140.2 Mb] [present study]. The 3.0 Mb [131.7–134.7 Mb] overlapping region of all three 9q34 duplications is denoted as "Region of overlap." The duplications in the patients from DECIPHER are denoted as 4 [P253579; age 17, 46,XX] [132.3–133.5 Mb], 5 [P254131; age 2, 46,XX] [133.7–140.1 Mb], and 6 [P255167; age 2, 46,XY] [133.7–140.1 Mb].

features were common to both patients in these reports: hypotonia, intellectual disability, developmental delay, characteristic head and facial features associated with dolichocephaly, facial asymmetry, narrow palpebral fissures, deep-set eyes, long nose, prominent chin, microstomia, microretrognathia, and characteristic features of the extremities, including long thin fingers and toes and camptodactyly (Table I). Gawlik-Kuklinska et al. [2007] concluded a 7.4 Mb (127.3–134.7 Mb) duplicated region in their patient was critical for the phenotypes they observed (Fig. 4). Like these two previously reported cases, our patients also exhibited hypotonia, mild intellectual disability, developmental delay, microstomia, microretrognathia, and long thin fingers and toes. Thus, the 3.0 Mb region (131.7–134.7 Mb) of 9q34.13-q34.3 that overlapped in the cases reported by previous studies [Gawlik-Kuklinska et al., 2007; Youngs et al., 2010], and in our patients is most likely associated with the manifestation of the phenotypes observed in all four patients (Fig. 4, Table I). Unlike the other patients, our patients did not have dolichocephaly, facial asymmetry, narrow palpebral fissures, deep-set eyes, or long nose. The locus or loci associated with these phenotypes may be located in a region (127.3–131.7 Mb) that is proximal to the overlapping region (Fig. 4, Table I). Our patients exhibited other characteristic facial features, such as round faces, hypertelorism, almond-shaped palpebral fissures, telecanthus, and short nose; those were not observed in the previously reported cases (Table I). The distal-most segment of 9q34 (134.7–140.2 Mb) in our patients is the strongest candidate for the origin of these phenotypes (Fig. 4). However, these phenotypes were not observed in Patient 2 [Youngs et al., 2010], who had the same 9qter duplication. Therefore, the duplication of the proximal segment (127.3–131.7 Mb) of the overlapping region may have more impact on facial appearance than the duplication of the distal segment of the overlapping region. Clinical analyses of more patients with 9qter duplication (134.7–140.2 Mb) are necessary to determine the phenotypes caused by duplication of this region. It should be noted that DECIPHER (Database of Chromosomal Imbalance and Phenotype in Human using Ensembl Resources) includes two patients (P254131 and P255167) with the same 9q34.2-qter duplication (133.7–140.1 Mb) and heterozygous deletion of 17pter (0.01–0.41 Mb) (Fig. 4, numbers 5, 6). These patients exhibited hypotonia (non-myopathic), intellectual disability, developmental delay, patchy café au lait pigmentation spots on the skin, and speech delay. The heterozygous 17pter 0.4 Mb deletion has not been reported to cause any diseases, including intellectual disability. Another patient (P253579) presenting with facial abnormality, intellectual disability, and developmental delay had a 9q34.1-q34.2 duplication (132.3–133.5 Mb) in the 3.0 Mb overlapping region (Fig. 4, number 4). Notably, these two duplicated regions are included in the duplicated region in our patients, but they do not overlap with each other. These findings suggest the following correlations between duplicated chromosomal segments of 9q34 and phenotypes: (1) two duplicated segments (133.7–140.1 and 132.3–133.5 Mb) in 9q34 are associated with intellectual disability and developmental delay; and (2) the locus or loci associated with characteristic facial appearance may be within a duplicated region of 1.2 Mb (132.3–133.5 Mb), even though the detailed clinical features of P253579 are not available. Of the 18 genes that map to this 1.2 Mb region, individual duplications of 12 genes are reported in the Database of Genomic Variants (DGV; found in normal population). Thus, increased copy number of one or more of the other six genes (*FUBP3*, *EXOSC2*, *ABL1*, *NUP214*, *FAM78A*, and *PPAPDC3*) in this region could be the cause of the intellectual disability, developmental delay, and characteristic facial appearance observed in our patients and P253579.

Chromosomal rearrangements, arising from unequal recombination between repeated sequences, are found in a subset of patients with autism spectrum disorder [Marshall et al., 2008]. Abnormal behaviors, including hyperactive behavior [Allderdice et al., 1983], food-seeking behavior [Gawlik-Kuklinska et al., 2007], hyperactivity, attention deficit disorders, and atypical autism [Youngs et al., 2010], were also reported in some patients with 9q34 duplication. Unlike these patients, our patients exhibited friendly and affectionate social behaviors and did not exhibit autistic features or attention deficit disorder. It is important to repeatedly monitor the behaviors

of our patients to determine whether the 9q34.1-qter duplication is associated with abnormal behaviors. In summary, our findings indicate that the duplication of 9q34 is a heterogeneous clinical condition and duplications of different segments of 9q34 are associated with a variety of symptoms. Genomic and clinical analyses of more patients carrying 9q34 duplications are necessary to better characterize the correlation between clinical phenotypes and specific 9q34 loci.

ACKNOWLEDGMENTS

We are grateful to the patients who participated in this study and their families. This study was supported by the Takeda Science Foundation (to N.W.) and the Health Labour Sciences Research Grant (to S.M. and N.W.).

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RESEARCH ARTICLE

AMERICAN JOURNAL OF medical genetics PART A

Craniofacial and Oral Features of Sotos Syndrome: Differences in Patients With Submicroscopic Deletion and Mutation of *NSD1* Gene

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Received 7 October 2010; Accepted 22 January 2011

Sotos syndrome is a well-known overgrowth syndrome caused by haploinsufficiency of *NSD1* gene located at 5q35. There are two types of mutations that cause *NSD1* haploinsufficiency: mutations within the *NSD1* gene (mutation type) and a 5q35 submicroscopic deletion encompassing the entire *NSD1* gene (deletion type). We investigated detailed craniofacial, dental, and oral findings in five patients with deletion type, and three patients with mutation type Sotos syndrome. All eight patients had a high palate, excessive tooth wear, crowding, and all but one patient had hypodontia and deep bite. Hypodontia was exclusively observed in the second premolars, and there were no differences between the deletion and mutation types in the number of missing teeth. Another feature frequently seen in common with both types was maxillary recession. Findings seen more frequently and more pronounced in deletion-type than in mutation-type included mandibular recession, scissors or posterior cross bite, and small dental arch with labioclination of the maxillary central incisors. It is noteworthy that although either scissors bite or cross bite was present in all of the deletion-type patients, neither of these was observed in mutation-type patients. Other features seen in a few patients include enamel hypoplasia (two deletion patients), and ectopic tooth eruption (one deletion and one mutation patients). Our study suggests that Sotos syndrome patients should be observed closely for possible dental and oral complications especially for malocculusion in the deletion-type patients.

Key words: Sotos syndrome; *NSD1*; submicroscopic deletion; small dental arch; malocclusion; mandibular recession

How to Cite this Article:
Hirai N, Matsune K, Ohashi H. 2011. Craniofacial and oral features of Sotos syndrome: differences in patients with submicroscopic deletion and mutation of *NSD1* gene.
Am J Med Genet Part A 155:2933–2939.

INTRODUCTION

Sotos syndrome is a congenital genetic disorder characterized by overgrowth starting before birth, specific facial manifestations (macrocephaly, prominent forehead, hypertelorism, downslanting palpebral fissures, and pointed chin), advanced bone age, and developmental impairment. Since its initial description by Sotos et al. [1964] several hundred patients have been reported to date. It may be accompanied by a variety of complications, including cardiovascular, urinogenital, and ophthalmic malformations, skeletal abnormalities, and seizures. Dental and oral findings have been reported to include premature tooth eruption, hypodontia, enamel hypoplasia, excessive tooth wear, maxillary and mandibular recession, talon cusps, fused teeth, and expanded pulp cavity of deciduous teeth [Welbury and Fletcher, 1988; Cole and Hughes, 1994; Inokuchi et al., 2001; Gomes-Silva et al., 2006; Takei et al., 2007; Nishimura et al., 2008].

Kurotaki et al. [2002] reported that this syndrome is caused by haploinsufficiency of the *NSD1* nuclear receptor SET domain containing protein 1 gene located on 5q35. There are two main types that cause *NSD1* haploinsufficiency: mutations within the *NSD1* gene, and a submicroscopic deletion in the region that contains the *NSD1* gene (constant deletion of approximately 2.2 Mb including *NSD1* and around 20 neighboring genes) [Kurotaki et al., 2002]. Nagai et al. [2003] investigated differences in clinical manifestations between these two types, and reported

Grant sponsor: Ministry of Education, Culture, Sports, Science and Technology (MEXT); Grant number: 2008-2012; Grant sponsor: Ministry of Health, Labour and Welfare, Japan.
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Published online 19 October 2011 in Wiley Online Library (wileyonlinelibrary.com).
DOI 10.1002/ajmg.a.33969

that major anomalies such as central nervous, cardiovascular, and urinogenital abnormalities are more common in the deletion-type. Their only reference to dental findings, however, stated that early tooth eruption occurred in both types with no significant difference.

The first detailed investigation of dental and oral findings seen in Sotos syndrome based on *NSD1* genetic diagnosis was carried out by Kotilainen et al. [2009]. They analyzed dental and oral findings from 13 patients with Sotos syndrome (all except one with the mutation type), including panoramic imaging, and reported the characteristic oral complications of Sotos syndrome, including hypodontia of the second premolars. We here report on the results of our investigation of detailed craniofacial, dental, and oral findings in five patients with deletion-type, and three patients with mutation-type Sotos syndrome.

MATERIALS AND METHODS

Patients

The eight patients comprised a group who underwent examination at Saitama Children's Medical Center. Five patients (three males, two females; age, 6–13 years) were identified as having a submicroscopic deletion on 5q35 including the *NSD1* gene, and three (all females; age, 6–10 years) were identified as having a mutation of the *NSD1* gene. Deletions were identified by fluorescence in situ hybridization (FISH) analysis of metaphase chromosomes from peripheral blood, using a total of seven bacterial artificial chromosome (BAC) clones comprising the BAC clone that includes the *NSD1* gene (RP11-99N22) together with those toward the centromere (RP11-880A16, RP11-690I8, RP11-991B23) and toward the telomere (RP11-147K7, RP11-452O4, and RP11-158F10). The results showed that the same ~2 Mb deletion was present in all five patients. Mutation analysis using genomic DNA extracted from peripheral blood was performed by polymerase chain reaction (PCR) and direct sequencing of all translated regions for exon 2–23. The results identified mutations generating premature termination in both Patients 6 and 7, comprising a five base deletion (2053–2057delAAGTA) and a base deletion (5431delC), respectively, and a missense mutation (4991G>C) in Patient 8. Details of clinical manifestations are shown in Table I. This study protocol was approved by the Ethics Committee of Saitama Children's Medical Center and proper informed consents were obtained from the legal guardians of the patients.

Oral and Dental Studies

Physical examination and dental cast studies were used to evaluate palatal morphology, tooth calcification, dental arches, occlusion, tooth size, and tooth eruption status. Panoramic and lateral cephalometric radiographs reconstructed from multi-detector row computed tomography (MDCT) were also used to evaluate the relationship of craniofacial, dental and skeletal structures, and hypodontia [Hirai et al., 2010; Yamauchi et al., 2010]. Crown and

TABLE I. Clinical Manifestations of Eight Patients With Sotos Syndrome

	Deletion type patients					Mutation type patients		
	1	2	3	4	5	6	7	8
Gender	M	F	F	M	M	F	F	F
Ages (years)	7	8	6	7	13	7	10	6
Overgrowth	–	–	–	–	–	+	+	+
Intellectual disability	Moderate	Moderate	Moderate	Moderate	Moderate	Mild	–	Mild
Seizure	–	+	–	+	–	–	+	–
Craniofacial features								
Macrocephaly	+	+	+	–	+	+	+	–
Prominent forehead	+	+	+	+	+	+	+	–
Hypertelorism	+	+	+	+	+	+	+	+
Downslanting palpebral fissures	+	+	+	+	+	+	+	+
Pointed chin	+	+	+	+	+	+	+	+
Strabismus	+	+	+	–	–	+	–	–
Skeletal anomaly								
Scoliosis	–	–	–	+	+	–	+	+
Pes planovalgus	+	+	+	+	+	+	–	+
Cardiovascular anomaly	AR	PDA	–	PDA, ASD, VSD	–	VSD, CoA	MR	–
Urogenital anomaly	Hydronephrosis, VUR	–	–	–	–	Urethrocele	Hydronephrosis, hydroureter	–
Others	Hearing loss	Myelomeningocele, umblical hernia	–	–	–	–	–	–

M, male; F, female; AR, aortic regurgitation; PDA, patent ductus arteriosus; ASD, atrial septal defect; VSD, ventricular septal defect, CoA, coarctation of aorta; MR, mitral regurgitation; VUR, vesicoureteral reflux; +, present; –, absent.

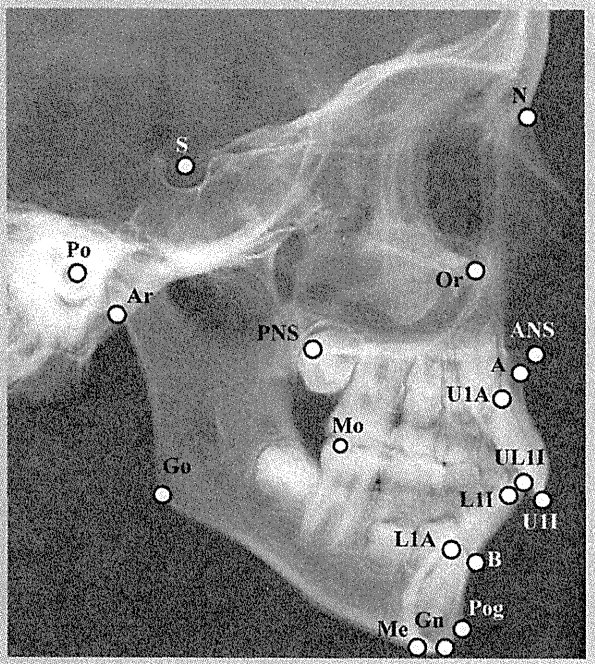

FIG. 1. Landmark points, angles, and lines used in cephalometoric analysis and profilogram. Landmarks: N, nasion; Or, orbitale; S, sella turcia; Po, porion; Ar, articulare; Go, gonion; Me, menton; Gn, gnathion; Pog, pogonion; A, A-point; B, B-point; ANS, anterior nasal spine; Mo, molar occlusion; U1A, upper central incisor root apex; U1I, upper central incisor edge; L1A, lower central incisor root apex; L1I, lower central incisor edge; UL1I, middle of U1I and L1I. Angles: convexity, N-A line to the A-Pog line; A-B plane, N-Pog line to the A-B line; SNA, S-N line to the N-A line; SNB, S-N line to the N-B line; facial angle, Po-Or line to the N-Pog line; SNP, S-N line to the N-Pog line; *y*-axis, Po-Or line to the S-Gn line; SN-S-Gn, S-N line to the S-Gn line; mandibular plane, Po-Or line to the Me-the lower border of the mandible line; gonial angle, Ar-the posterior border of the ramus of the mandible line to the Me-the lower border of the mandible line; GZN, S-N line to the Ar-the posterior border of the ramus of the mandible line; FH to SN, Po-or line to the S-N line; U-1 to FH plane, U1I-U1A line to the Po-or line; U-1 to SN plane, U1I-U1A line to the S-N line; L-1 to mandibular, L1I-L1A line to the Me-the lower border of the mandible; interincisal, U1I-U1A line to the L1I-L1A line; occlusal plane, Po-Or line to the Mo-UL1I line.

dental arch sizes were measured using a caliper with a resolution accuracy of 0.01 mm. Lateral cephalometric analysis was performed based on the method developed by Iizuka and Ishikawa [1957] (Fig. 1). All data in this study (tooth size, dental arch form size, and cephalometric findings) were compared with standard values for Japanese individuals [Otsubo, 1957; Otsubo et al., 1964].

RESULTS

Oral and dental anomalies noted in eight patients are summarized in Table II. All eight patients had a high palate, crowding, and excessive tooth wear. All but one (Patient 1 with *NSD1* deletion) had hypodontia exclusively in the second premolars. There were no differences between the deletion-type and mutation-types in the number of missing teeth (mean number of missing teeth was 2 in the deletion-type and 2.6 in the mutation-type) (Fig. 2). The results of cephalometric analysis showed that among the five deletion-type patients, maxillary and mandibular recession was present in three and maxillary recession alone in one, whereas among the three mutation-type patients maxillary and mandibular recession was present in one and maxillary recession alone in one. The deletion-type was regarded as having a stronger tendency for mandibular recession (Table III). In terms of occlusion, crowding was present in all patients, and deep bite was seen in all but one (Patient 2 with *NSD1* deletion). It is noteworthy that although either scissors bite (Patients 1, 3, and 4) or cross bite (Patients 2 and 5) was present in all of the deletion-type patients, neither of these was observed in mutation-type patients (Fig. 3).

Small dental arch was present in all the deletion-type patients and one mutation-type patient (Table IV). In terms of morphological categories of small dental arch, the maxilla exhibited a narrow dental arch with labioclination of the central incisors in all five deletion-type patients, with the mandible being saddle-shaped in three patients and U-shaped in two, while the mutation-type patient had U-shaped upper and lower dental arches (Fig. 4). In terms of tooth size, both microdontia and macrodontia were occasionally seen in both the deletion-type and mutation-types, but no characteristic findings were present in either type (data not shown). Enamel hypoplasia was present in two out of the five deletion-type patients (Patients 2 and 3), but was not present in the mutation-type. In addition, ectopic eruption of the first molar was present in one deletion-type patient (Patient 4, right mandibular) and one mutation-type patient (Patient 6, bilateral maxillary). Some representative photographs of oral and dental anomalies noted in patients studied are shown in Figure 5.

DISCUSSION

The oral manifestations observed in common with both deletion and mutation type Sotos syndrome patients noted here were a high palate, excessive tooth wear, recession of maxilla, deep bite, crowding, and hypodontia. Hypodontia has been previously described by several authors [Inokuchi et al., 2001; Callnan et al., 2006; Gomes-Silva et al., 2006; Nishimura et al., 2008]. Kotilainen et al. [2009] recently investigated 13 patients with Sotos syndrome (12 patients with *NSD1* mutations and one with *NSD1* deletion) and found one or more premolar teeth were absent in 9 out of 13 patients (8 out of 12 mutation patients and one deletion-type patient). Based on the observation that the deletion patient had the most severe phenotype of tooth agenesis, involving not only the second premolars and the third molars, but also one mandibular incisor, they noted the possibility that patient with the *NSD1* deletion had the most severe tooth agenesis. In our study, however, which included five deletion-type patients, although similar high rates of hypodontia were observed in both the deletion-type and mutation-type, we did not observe any difference in severity in either the deletion-type or mutation-type.

One noteworthy difference between the deletion-type and mutation-type was the fact that either scissors bite or cross bite

TABLE II. Oral and Dental Anomalies in Eight Patients

	Deletion type patients					Mutation type patients			Total	
Oral anomalies	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**Deletion type**	**Mutation type**
High palate	+	+	+	+	+	+	+	+	5/5	3/3
Excessive tooth wear	+	+	+	+	+	+	+	+	5/5	3/3
Hypodontia	−	+	+	+	+	+	+	+	4/5	3/3
Maxillary recession	+	−	+	+	+	+	+	−	4/5	2/3
Mandibular recession	−	−	+	+	+	−	+	−	3/5	1/3
Malocclusion										
Scissors bite	+	−	+	+	−	−	−	−	3/5	0/3
Cross bite	−	+	−	−	+	−	−	−	2/5	0/3
Deep bite	+	−	+	+	+	+	+	+	4/5	3/3
Crowding	+	+	+	+	+	+	+	+	5/5	3/3
Small dental arch	+	+	+	+	+	−	+	−	5/5	1/3
Maxilla	N	N	N	N	N	U	U	U		
Mandibula	S	U	S	S	U	U	U	U		
Labioclination of maxillary central incisor	+	+	+	+	+	−	−	−	5/5	0/3
Enamel hypoplasia	−	+	+	−	−	−	−	−	2/5	0/3
Ectopic tooth eruption	−	−	−	+	−	+	−	−	1/5	1/3

N, narrow dental arch; U, U-shaped dental arch; S, saddle-shaped dental arch; +, present; −, absent.

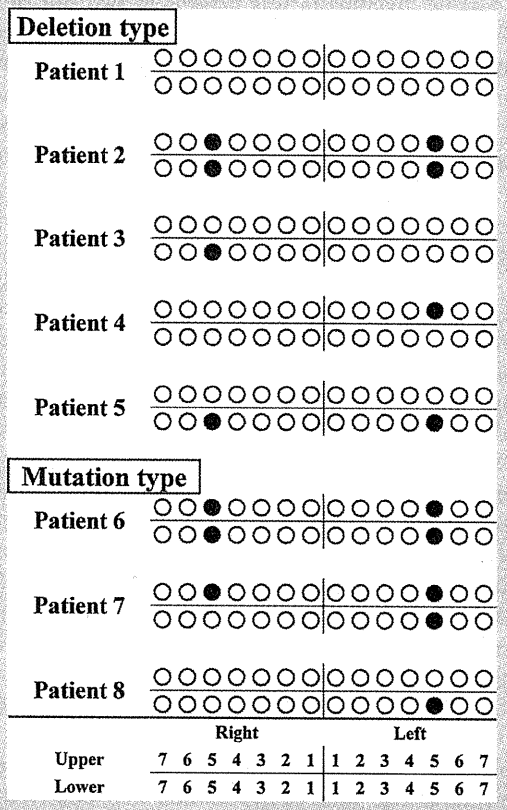

FIG. 2. Hypodontia in eight patients with Sotos syndrome. •, Congenitally missing teeth.

was observed in all deletion-type patients, whereas neither was present in the mutation-type. All of the deletion-type patients had small dental arches, and in terms of morphological categories, the maxilla exhibited a narrow dental arch with labioclination of the central incisors in all five deletion-type patients, with the mandible being saddle-shaped in three patients and U-shaped in two. Only one single mutation-type patient had small dental arches with both upper and lower dental arches being U-shaped. Narrowing of the dental arch is more pronounced in the narrow-shape and saddle-shape compared with the U-shape. It is possible that the degree of narrowing of the dental arch in the deletion-type and the misalignment in arch morphology between the maxilla and mandible causes scissors bite or cross bite.

In addition, maxillary and mandibular recession has also been reported as a dental manifestation of Sotos syndrome [Welbury and Fletcher, 1988; Takei et al., 2007]. In our results, there was a tendency toward maxillary and mandibular recession in the deletion-type and maxillary recession in the mutation-type. Based on these findings, there was a tendency for maxillary recession to occur in both the deletion-type and mutation-type, but there was also a tendency toward the occurrence of mandibular recession in the deletion-type. Taken in conjunction with the pronounced mandibular recession seen in the deletion-type on cephalometric analysis, mandibular malformations, including those of the dental arch, may be regarded as characteristic of the deletion-type. The cause is unknown, but in the deletion-type, minute genome imbalances, involving considerable number of genes other than the *NSD1* gene, may either: (1) directly cause deficient growth of the mandibular area; or (2) secondarily cause malocclusion or abnormal dental arch morphology as a result of dysfunction of the perioral muscles associated with more

TABLE III. Lateral Cephalometric Analysis With MDCT of Eight Patients

	Deletion type patients					Mutation type patients		
	1	2	3	4	5	6	7	8
Skeletal								
Covexity	−2.56	−1.05	−0.66	−1.80	−1.95	−4.56	−2.52	−2.94
A–B plane	−1.12	−3.96	1.68	−0.32	1.40	2.15	1.96	2.56
SNA	−2.54	1.24	−2.28	−3.45	−2.32	−2.69	−3.18	−1.63
SNB	−1.80	1.71	−3.31	−3.18	−2.84	−0.76	−2.29	−0.07
Facial angle	0.68	−3.23	−0.47	0.38	0.34	0.38	−1.55	1.43
SNP	−0.71	1.77	−1.76	−1.16	−0.63	0.02	−2.22	0.11
Y-axis	−0.50	−0.37	−0.38	−0.08	0.17	−0.36	1.29	−1.34
SN-S-Gn	3.08	−1.30	0.32	3.86	0.93	0.22	1.86	−0.30
Mandibular plane	1.29	1.05	−1.47	0.30	1.12	−0.58	1.98	−0.29
Gonial angle	6.24	−0.15	−4.06	−5.27	0.73	0.29	0.44	3.06
GZN	0.77	0.09	3.01	2.84	1.19	−0.37	1.52	−0.27
FH to SN	2.38	−1.15	0.71	2.55	0.98	0.52	0.97	1.37
Denture								
U-1 to FH plane	1.30	1.37	2.94	0.30	0.72	−0.47	0.50	0.69
U-1 to SN plane	0.51	1.87	2.56	−0.52	0.24	−0.64	−0.46	0.21
L-1 to mandibular	−2.24	−0.47	0.98	−1.47	−1.64	−2.39	−0.46	−1.93
Interincisal	−0.07	−1.07	−1.53	0.46	0.22	1.90	−0.83	0.71
Occlusal plane	−0.99	1.09	−0.76	−0.24	2.97	−1.37	1.35	−0.49

Unit, SD.

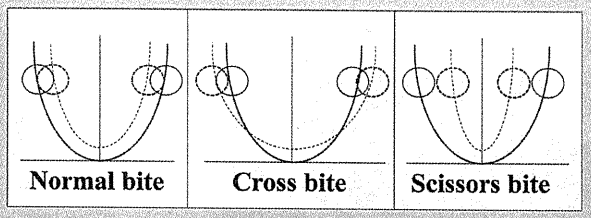

FIG. 3. Schematic representations of normal and abnormal occlusions. ———, Maxillary dental arch; - - - - - - -, mandibular dental arch; ◯, maxillary first molar; ◌, mandibular first molar.

pronounced developmental impairment [Grabowski et al., 2007a,b; Stahl et al., 2007].

Enamel hypoplasia has also been reported as a dental manifestation of Sotos syndrome [Inokuchi et al., 2001]. Kotilainen et al. [2009] reported enamel hypoplasia in four out of 13 patients (all mutation type). In our study, enamel hypoplasia was present in two out of five deletion-type patients, but not in any mutation-type patients. Enamel hypoplasia is thought to be a common manifestation that can occasionally occur in both the deletion-type and mutation-type rather than a manifestation that is prone to occur in either type.

As mild to moderate intellectual disability is common in Sotos syndrome, conventional panoramic, and cephalometric studies

TABLE IV. Dental Arch Measurements in Eight Patients

	Deletion type patients					Mutation type patients		
	1	2	3	4	5	6	7	8
Maxillary								
W_c	−0.55	Deciduous	0.04	Deciduous	1.79	−1.75	0.61	−1.30
W_6	−3.76	−3.59	0.02	−4.18	−3.11	−1.73	−2.04	−1.76
L_{16}	2.68	1.33	2.88	1.02	−0.69	1.44	0.14	−1.36
Mandibular								
W_c	Deciduous	Deciduous	−1.00	Deciduous	−0.73	−1.81	−2.70	−0.30
W_6	−4.82	−2.51	−2.16	−4.45	−3.38	−1.57	−4.34	0.96
L_{16}	0.81	1.32	1.85	0.63	−3.40	0.20	−2.07	0.56

Unit, SD.
The W_c and W_6 represent the distance between the primary cuspids (the cuspids), and the first molars, respectively. The L_{16} represents the length from the mesial surface of the first molars to central point of incisors.

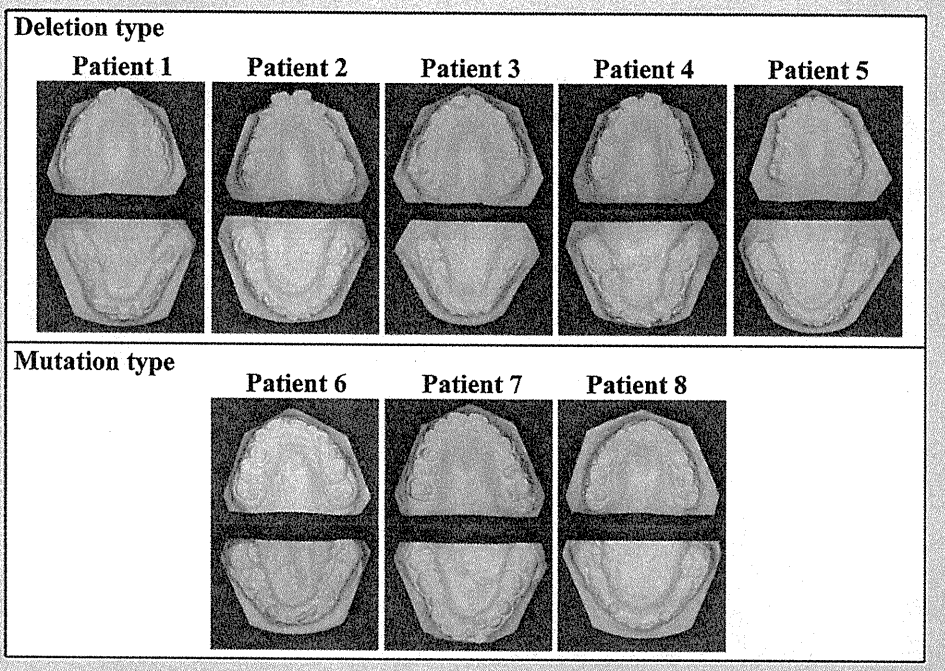

FIG. 4. Dental arch shapes of eight patients. Upper panel: maxillary dental casts, lower panel: mandibular dental casts. A narrow maxillary dental arch with labioclination of the central incisors is noted in all five deletion type patients, with the mandibula being saddle-shaped in three patients (Patients 1, 3, and 4) and U-shaped in two (Patients 2 and 5), while U-shaped upper and lower dental arches are noted in all three mutation-type patients (Patients 6, 7, and 8).

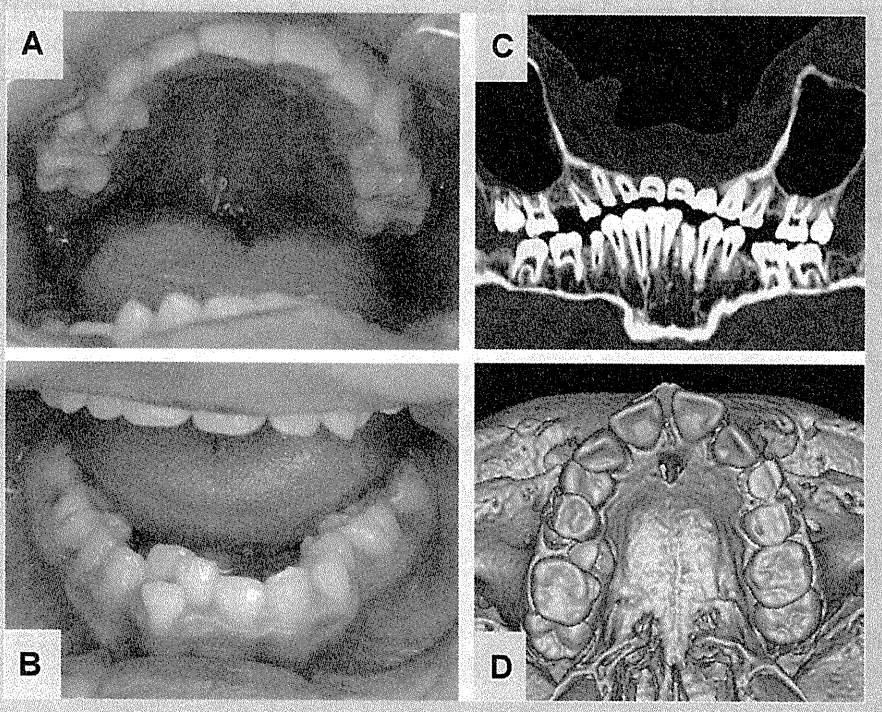

FIG. 5. Oral photographs (A,B) and MDCT-synthesized panoramic radiograph (C) of Patient 7 at age of 10 years and MDCT-synthesized upper dental arch of Patient 6 at age of 7 years (D). Note: high palate, malocclusion, small dental arch, excessive tooth wear (A,B), missing upper second premolars on both side and lower left second premolar (C), ectopic tooth eruption of first molars on both side (D).

were often difficult to perform in childhood. Thus, in this study, MDCT was used as a substitute for cephalometric radiographs and panoramic radiographs, and by which maxillofacial manifestations could be accurately evaluated [Hirai et al., 2010; Yamauchi et al., 2010].

In view of oral and dental management, we would like to provide recommendations as follows: periodic dental check up to prevent dental caries or gingivitis should be started early after one or more deciduous teeth have erupted. Around age 7 years, detailed oral and dental evaluations, including dental cast studies and MDCT, is recommended for possible hypodontia and malocclusion. If the patient has hypodontia, preceding deciduous tooth (teeth) should be maintained as long as possible with proper care. Although malocclusion like scissors bite and cross bite requires early treatment, including expansion of upper or lower jaw, to prevent craniofacial disabilities such as facial asymmetry and temporomandibular joint dysfunction, the treatment should be carefully decided based on consideration of capability of cooperation of the patients.

In conclusion, features seen more frequently and more pronounced form in deletion-type than in mutation-type were small dental arch with labioclination of the maxillary central incisors, mandibular recession, and scissors or posterior cross bite. Sotos syndrome patients should be followed closely for possible dental and oral complications especially for malocculusion in the deletion-type.

ACKNOWLEDGMENTS

The authors are grateful to Prof. Takahide Maeda for his helpful advice. We also thank to Dr. Kenji Shimizu, Dr. Yasuo Takahashi, and Hitoshi Yabe for their invaluable assistance. This study was funded in part by a Grant for the Support of Projects for Strategic Research at Private Universities by the Ministry of Education, Culture, Sports, Science and Technology (MEXT; 2008-2012), and by a grant from the Ministry of Health, Labour and Welfare, Japan.

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Brain & Development 33 (2011) 576–579

BRAIN & DEVELOPMENT
Official Journal of the Japanese Society of Child Neurology

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Original article

A familial case of LEOPARD syndrome associated with a high-functioning autism spectrum disorder

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Received 6 July 2010; received in revised form 3 October 2010; accepted 6 October 2010

Abstract

A connection between LEOPARD syndrome (a rare autosomal dominant disorder) and autism spectrum disorders (ASDs) may exist. Of four related individuals (father and three sons) with LEOPARD syndrome, all patients exhibited clinical symptoms consistent with ASDs. Findings included aggressive behavior and impairment of social interaction, communication, and range of interests. The coexistence of LEOPARD syndrome and ASDs in the related individuals may be an incidental familial event or indicative that ASDs is associated with LEOPARD syndrome. There have been no other independent reports of the association of LEOPARD syndrome and ASDs. Molecular and biochemical mechanisms that may suggest a connection between LEOPARD syndrome and ASDs are discussed.

Keywords: LEOPARD syndrome, Noonan syndrome; Autism spectrum disorders (ASDs); RAS/MAPK signal transduction pathway

1. Introduction

LEOPARD syndrome (OMIM#151100) is a rare autosomal dominant disorder characterized by Lentigines, Electrocardiogram abnormalities, Ocular hypertelorism, Pulmonic valvular stenosis, Abnormalities of genitalia, Retardation of growth, and Deafness. This syndrome is caused by germline missense mutations in the *PTPN11* gene that encodes Src homology 2 domain-containing tyrosine phosphatase 2 (Shp2): non-receptor protein-tyrosine phosphatase comprising two N-terminal SH2 domains, a catalytic domain, and a C terminus with tyrosylphosphorylation sites and a proline-rich stretch. The mutations induce catalytically impaired Shp2 by a "dominant negative effect" [1–2].

In the more common Noonan syndrome, approximately 50% of patients have *PTPN11* mutations scattered over the entire Shp2, including the catalytic domain. The mutations resulting in the Noonan phenotype are the "gain-of-function" mutations, and they exhibit substantially increased catalytic ability. Although LEOPARD syndrome and Noonan syndrome are caused by *PTPN11* mutations resulting in opposite effects, they share many common clinical features, including physical dysmorphic findings and intellectual disability [1].

The term "autism spectrum disorders (ASDs)" was first used by Lorna Wing [3] and then widely used as a category comprised of autistic disorder, Asperger's

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doi:10.1016/j.braindev.2010.10.006

disorder, and other related conditions [4]. These conditions are very common neurobehavioral disorders that are characterized by impairments in three behavioral domains, including social interaction, language/communication/imaginative play, and a range of interests and activities [3–5].

At least ten genes have been reported to be associated with ASDs [6]. Except for Rett syndrome, the other pervasive developmental disorder (PDD) subtypes including autistic disorder, Asperger's disorder, disintegrative disorder, and PDD Not Otherwise Specified (PDDNOS) are not tightly linked to any particular gene mutations. Several common genetic syndromes are known to be associated with ASDs. Autism is frequent in patients with tuberous sclerosis (TSC) [7], with neurofibromatosis type 1 [8,9] and with Fragile X syndrome [10]. Studies of psychological profiles of adults with Noonan syndrome did not suggest a specific behavioral phenotype, but difficulties with social competence and emotional perceptions were noted [11]. A case of Noonan syndrome who was also diagnosed with autism was reported [12]. The present study of neuropsychiatric evaluation in a familial case of LEOPARD syndrome indicates all patients satisfied the criteria of ASDs. An association of LEOPARD syndrome and ASDs has not been reported previously. The familial case presented in this report may suggest such an association.

2. Patients and methods

After obtaining written informed consent, fifteen coding exons in *PTPN11* were sequenced in each patient following the methods described somewhere else [13].

Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV-TR) [5] and The high-functioning Autism Spectrum Screening Questionnaire (ASSQ) [14] were used in neuropsychiatric evaluation of the subjects.

Patient 1 is a 20-year-old male who was born as the second child to a non-consanguineous Japanese couple. His early developmental milestones were reportedly unremarkable. He was clinically diagnosed with LEOPARD syndrome at age 7 years based on findings that included lentigines, multiple café-au-lait spots, electrocardiogram (ECG) abnormalities, ventricular septal defect, ocular hypertelorism, short stature, and unilateral renal hypoplasia. *PTPN11* mutation analysis revealed a heterozygous mutation of 1403C > T (T468 M). The patient was diagnosed as having Asperger's disorder based on ASSQ and DSM-IV-TR, at age 12 years. His intelligence quotient (IQ) by the Wechsler Intelligence Scale for Children-third edition (WISC-III) was 85 (verbal: 77, performance 98). His ASSQ score by mother's rating was 41. He met the DSM-IV-TR diagnostic criteria of Asperger's disorder with all subcategories in the category of Qualitative impairment in social interaction (Category 1), three subcategories (1,2, and 4) in the category of Restricted repetitive and stereotyped patterns of behavior, interests and activities (Category 2), and the rest of the four categories (Table 1).

Patient 2 is a 15-year-old younger brother of Patient 1. His early infantile developmental milestones were unremarkable. He was diagnosed with growth retardation at age 2½ years. At age 12 years his clinical findings of a few café-au-lait spots, ocular hypertelorism, and undescended testes led us to obtain *PTPN11* mutation analysis, which showed the same heterozygous mutation of 1403C > T. At age 9 years, a diagnosis of Asperger's disorder was made based on ASSQ and DSM-IV-TR. His full-scale IQ by WISC-III at age 9 years was 99 (verbal 104, performance 92). His ASSQ score by parental rating was 32 at age 15 years. He also met the Asperger's disorder diagnostic criteria with all subcategories of Category 1, three of Category 2 (1, 2, and 4), and the rest of the categories (Table 1).

Patient 3 is the 22-year-old eldest brother of Patients 1 and 2. His developmental milestones were normal, although his ritualistic behavior and difficulties in relating to peers were noted in his childhood. He had a surgical repair of bilateral undescended testes and inguinal hernia. He was diagnosed with Wolff-Parkinson-White syndrome at age nine years. He has ocular hypertelorism and short stature. The same *PTPN11* heterozygous mutation found in the two younger siblings was identified in this patient. He attends college, and was diagnosed as having PDDNOS, because he also had impaired development of reciprocal social interaction associated with communication skills, repetitive routine, and ritualistic behavior. His ASSQ score was 7 at age 22 years (Table 1).

Patient 4 is a 55-year-old male who is the father of the siblings. He has prominent lentigines, bilateral mild hearing loss, cardiac anomalies, ECG abnormalities, short stature, and apparent ocular hypertelorism. His early developmental milestones are not well known. He has been noted to have obsession with a specific topic, repetitive routine and rituals, and clumsy movements. At age 50 years, his social skills and aggressive behavior were noted to be deteriorating, and consequently he was suspected of having Asperger's disorder based on DSM-IV-TR. He met the diagnostic criteria of Asperger's disorder with Category 1 (1 and 3), Category 2 (1 and 2), and the rest of the four categories. His ASSQ score was 20 at age 55 years by his wife's evaluation. He has the same heterozygous *PTPN11* mutation (Table 1).

3. Discussion

The presented familial case of LEOPARD syndrome included individuals (patients 1, 2, and 4) diagnosed with or suspected of having Asperger's disorder, and

Table 1
Summary of clinical findings and *PTPN11* mutation.

	Pt. 1 Male	Pt. 2 Male	Pt. 3 Male	Pt. 4Male
Age	20 y	15 y	22 y	55 y
Physical findings				
Skin: café-au-lait spots	multiple	a few	a few	a few
Lentigines	+++	+++	-	+++
Cardiac defects	VSD	No	No	No
EKG abnormalities	+	No	WPW	No
Ocular hypertelorism	+	+	+	+
Pulmonary stenosis	No	No	No	No
Abnormal genitalia	No	Und. Testes*	Und. Testes*	No
Renal anomalies	R-hypoplasia	No	No	No
Retardation of growth	Yes	+	+	No
Deafness	No	No	No	Yes
Miscellaneous:				
Rocker bottom feet	Yes	Yes	Yes	No
Macrocephaly	Yes	Yes	Yes	No
PTPN11 mutation	T468 M	T468 M	T468 M	T468 M
Neuropsychological				
Diagnosis	AD**	AD**	PDDNOS***	AD**
ASSQ score[1] (age)	41 (12 y)	32 (15 y)	7 (22 y)	20 (50 y)
WISC-III[2] (age)	85 (12 y)	99 (9 y)	n/a	n/a
-Verbal/performance	77/98	104/92	n/a	n/a

* Und. Testes, undescended testes.
** AD, Asperger's disorder.
*** PDDNOS, Pervasive developmental disorder not otherwise specified.
[1] ASSQ score, Autism Spectrum Screening Questionnaire Score. The cutoff score of 3 predicts 91% of the true positive rate of Autistic spectrum disorders.
[2] WISC-III, Wechsler Intelligence Scale for Children-third edition.

patient 3 was diagnosed as having PDDNOS, which may lead to the diagnosis of ASD. ASDs were first introduced by Lorna Wing, who suggested that Asperger's disorder is a type of ASD and described in detail its various manifestations in speech, nonverbal communication, social interaction, motor coordination, motor clumsiness, and idiosyncratic interests [3]. Patient 3 did not have enough clinical symptoms to meet the diagnostic criteria for Asperger's disorder; however, he had some symptoms suggestive of ASD in his childhood that led to a diagnosis of PDDNOS.

The ASSQ is a 27-item checklist for completion by lay informants when assessing characteristic symptoms of Asperger's disorder and high-functioning autism in children and adolescents with normal intelligence or mild mental retardation. The ASSQ allows for rating on a 3-point scale (0, 1, or 2; 0 indicating normality, 1 some abnormality, and 2 definite abnormality). The range of possible scores is 0–54. The mean ASSQ parent scores in the Asperger's disorder validation sample were 25.1 (SD, 7.3) [14]. The cutoff score of 13 is 91% of the true positive rate of ASDs. The ASSQ score was established as a screening tool primarily for children between 6 and 17 years of age by parents and/or teachers. The delayed evaluation of patient 3 may account for the difference in diagnosis between this patient and his siblings.

ASDs are known to be associated with particular genetic disorders such as fragile X syndrome [10,15,16], tuberous sclerosis (TSC) [7], and neurofibromatosis type 1 [8,9]. Fifty percent of children with TSC have behavioral problems in the form of ASDs. Gene mutations in either *TSC1* or *TSC2* influence neural precursors, resulting in abnormal cell differentiation and dysregulated control of cell size. These cells migrate to the cortex to generate an abnormal collection of inappropriately positioned neurons, causing widespread cortical disorganization and structural abnormalities [7]. Mutations in *PTPN11* causing LEOPARD syndrome induce catalytically impaired Shp2. In situ hybridization detected Shp2 expression in the neural ectoderm and nervous system in mouse embryos, suggesting an involvement of Shp2 in neural development. Shp2 is a critical signaling molecule in the coordinated regulation of progenitor cell proliferation and neuronal/astroglial cell differentiation. The studies with mutant mouse strains with Shp2 selectively deleted in neural precursor cells showed a dramatic phenotype of growth retardation, early postnatal lethality, and multiple defects in proliferation and cell fate specification in neural stem/progenitor cells [17]. The product of the TSC2 gene tuberin is known to up-regulate the B-RAF/MEK/MAPK signal transduction pathway. B-RAF is required for neuronal differentiation, suggesting another possible link between B-RAF signaling and the clinical manifestations of TSC including ASDs [18]. Disturbed neuronal cell differentiation and development due to mutations in