features, including a flat face, downslanting palpebral fissures, low-set posteriorly rotated ears, and a thin upper lip vermilion (Fig. 1). Hearing and visual acuity were normal. His weight was 9.3 kg (−1.9 SD), and his length was 82.6 cm (−0.9 SD). His head circumference was 46.5 cm (−1.1 SD). Routine laboratory tests were normal. Neuroradiological analysis revealed no significant abnormalities. Conventional cytogenetic examination of G-banded chromosomes from peripheral blood lymphocytes was normal. Microarray analyses were performed to reveal submicroscopic chromosomal aberrations.

Patient 2 (16p12.2–p11.2 Duplication)

The patient, a 9-year-old girl, was born to non-consanguineous healthy Japanese parents. Her family history was unremarkable. She was born at 38 weeks' gestation. Her birth weight was 3,200 g. Her gross development was delayed. She started to walk independently at 18 months old. Her development in speech and social interaction were retarded. At 4 years of age, she was diagnosed with ASD. G-banded karyotyping revealed a normal female karyotype. Brain MRI was normal. EEG showed epileptic discharges, but she was free from epileptic seizures. She visited our hospital for further medical studies. Her dysmorphic features included a round face, a flat nasal bridge, full cheeks, a wide philtrum, a wide mouth, a pointed chin, fetal pads on fingers and toes, and generalized hypertrichosis (Fig. 2). Her height, weight, and head circumference were 119 cm (−2.3 SD), 22.8 kg (−1.2 SD), and 54.2 cm (+1.5 SD), respectively. Relative macrocephaly was noted. She had difficulties with daily activities. In addition, impaired social interaction, poor social skills, and strict adherence to routine behaviors were noted. She was again diagnosed with ASD according to the DSM–VI criteria. Her DQ was 76 according to the standard Japanese method. Recently, her communication skill improved. Microarray analyses were performed to reveal submicroscopic chromosomal aberrations.

MATERIALS AND METHODS

With written informed consent, genomic DNA was obtained from blood leukocytes. A copy number analysis was performed using the

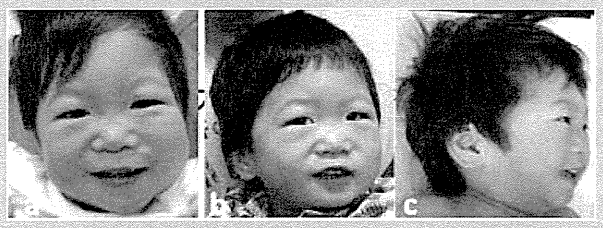

FIG. 1. a,b,c: Facial appearance of Patient 1 with the 16p12.2–p11.2 deletion at 2 years of age. He shows dysmorphic features, including a flat face, downslanting palpebral fissures, low-set posteriorly rotated ears, and a thin upper lip vermilion. (Permission for presentation has been obtained from his parents).

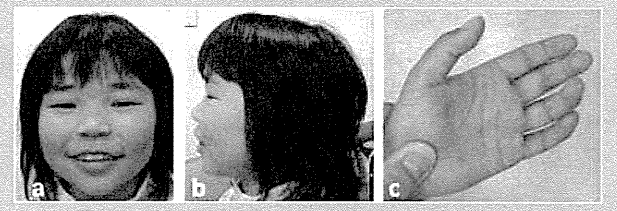

FIG. 2. a,b: Facial appearance of Patient 2 with the 16p12.2–p11.2 duplication. Her features included a round face, a flat nasal bridge, full cheeks, wide philtrum, a wide mouth, and a pointed chin. c: Fetal pads of the fingers were observed. (Permission for presentation has been obtained from her parents).

CytoScan HD Array (Affymetrix, Santa Clara, CA) which features 2.7 million genetic markers covering the whole human genome. Genomic DNA of the patients was diluted to 50 μg/μl and processed according to the protocol (Affymetrix Cytogenetics Copy Number Assay User Guide) supplied by Affymetrix. The data were analyzed with the Chromosome Analysis Suite software (Affymetrix).

RESULTS

The SNP array analysis confirmed aberrations on the short arm of chromosome 16. The karyotype of Patient 1 was 46, XY, arr 16p12.2p11.2(21,306,156–29,053,253) × 1 (hg19) (Fig. 3). The deletion size was 7.7 Mb. The segment contained OMIM disease genes (*OTOA*, *SCNN1G*, *SCNN1B*, *COG7*, *EARS2*, *PALB2*, *IL4R*, *IL21R*, *CLN3*, *TUFM*, *SH2B1*, *ATP2A1*, and *CD19*). The karyotype of Patient 2 was 46, XX, arr 16p12.2p11.2 (21,516,366–28,234,120) × 3 (hg19) (Fig. 4). The duplication size was 6.7 Mb. The segment contained OMIM disease genes (*OTOA*, *SCNN1G*, *SCNN1B*, *COG7*, *EARS2*, *PALB2*, *IL4R*, and *IL21R*). The duplicated region of Patient 2 was smaller than the deleted region of Patient 1. *SH2B1*, *ATP2A1*, and *CD19* were not included in the duplication. These copy number variations were confirmed by FISH analysis (Fig. 5). In both families, the parents did not have the abnormalities.

DISCUSSION

The chromosome 16p12.2–p11.2 deletion syndrome 7.1 to 8.7 Mb [MIM#613604] (21.4 to 29.3 Mb) is a new syndrome resulting from non-allelic homologous recombination. The deleted segment is distal to the 16p11.2 deletion syndrome region and is distinguished from the 16p11.2 deletion syndrome which is associated with autism [Kumar et al., 2008; Marshall et al., 2008].

Hernando et al. [2002] reported the first case of multiple congenital anomalies associated with a deletion of 16p11.2 confirmed by array CGH. The male infant showed severe intrauterine growth retardation and dysmorphic features. The patient died at the age of 5 months due to cardiac disease. Ballif et al. [2007] identified a recurrent de novo pericentromeric deletion of

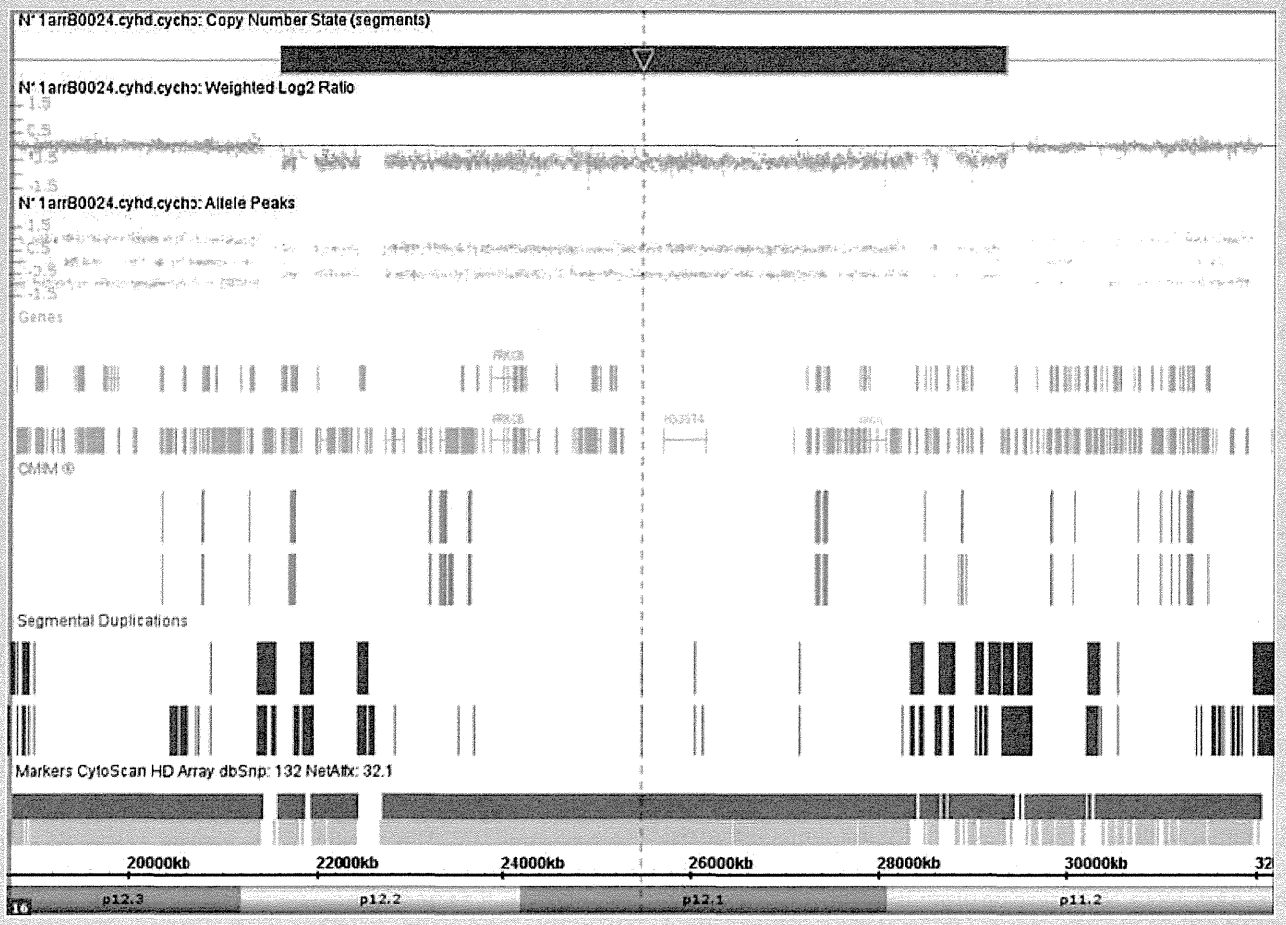

FIG. 3. Results of the SNP array analysis of Patient 1. The red bar indicates the 16p12.2–p11.2 deletion. The deletion size was 7.7 Mb. The segment contained OMIM disease genes (*OTOA, SCNN1G, SCNN1B, COG7, EARS2, PALB2, IL4R, IL21R, CLN3, TUFM, SH2B1, ATP2A1*, and *CD19*).

16p12.2–p11.2 in four individuals with developmental disabilities by array-CGH. The four patients had common clinical features. Battaglia et al. [2009] and Hempel et al. [2009] reported on further patients with a 16p12.2–p11.2 deletion.

Dysmorphic features including flat face, feeding difficulties, significant delay in speech development, and recurrent ear infections are common symptoms of the 16p12.2–p11.2 deletion syndrome. Some patients have anxiety, irritability, hyperactivity, short attention ability, and introverted behavior. However, ASD is not described in the patients. Patient 1 shared many of the clinical findings in these reports (Table I). Our result further confirmed that the 16p12.2–p11.2 deletion is responsible for a distinct multiple congenital anomalies and intellectual disability (MCA/ID) syndrome without autistic features.

The distal breakpoint of these patients was 21.4 Mb from the 16p telomere. The proximal breakpoints of these patients were 28.3–29.3 Mb from the 16p telomere and differed in the 16p11.2 region. The distal and the proximal breakpoint of Patient 1 was 21.3 Mb and 29.1 Mb from the 16p telomere, respectively.

Some disease-causing genes are included in the deleted region. Hempel et al. [2009] speculated that heterozygosity of the *OTOA* gene may cause hearing impairment in some patients. Herterozygous deletion of *CD19* may lead to recurrent ear infections. Patient 1 had recurrent ear infections. Routine laboratory investigations revealed no immunological abnormalities. Deletions encompassing the *SH2B1* gene were recently reported in early-onset obesity [Bochukova et al., 2010]. Although the *SH2B1* gene was deleted in Patient 1, he was not obese due to feeding difficulties. We should be careful for the later onset obesity.

Reciprocal duplications of 16p12.2–p11.2 have been reported in some patients with ASD and dysmorphic features [Engelen et al., 2002; Finelli et al., 2004]. However, the precise boundaries of the duplication were not defined because they were studied only by standard cytogenetic techniques. Tabet et al. [2012] reported two monozygotic twins carrying a de novo 16p12.2–p11.2 duplication of 8.95 Mb (21.28–30.23 Mb) encompassing both the 16p11.2 and 16p12.2–p11.2 regions. The twins exhibited ASD, severe intellectual disabilities, and dysmorphic features,

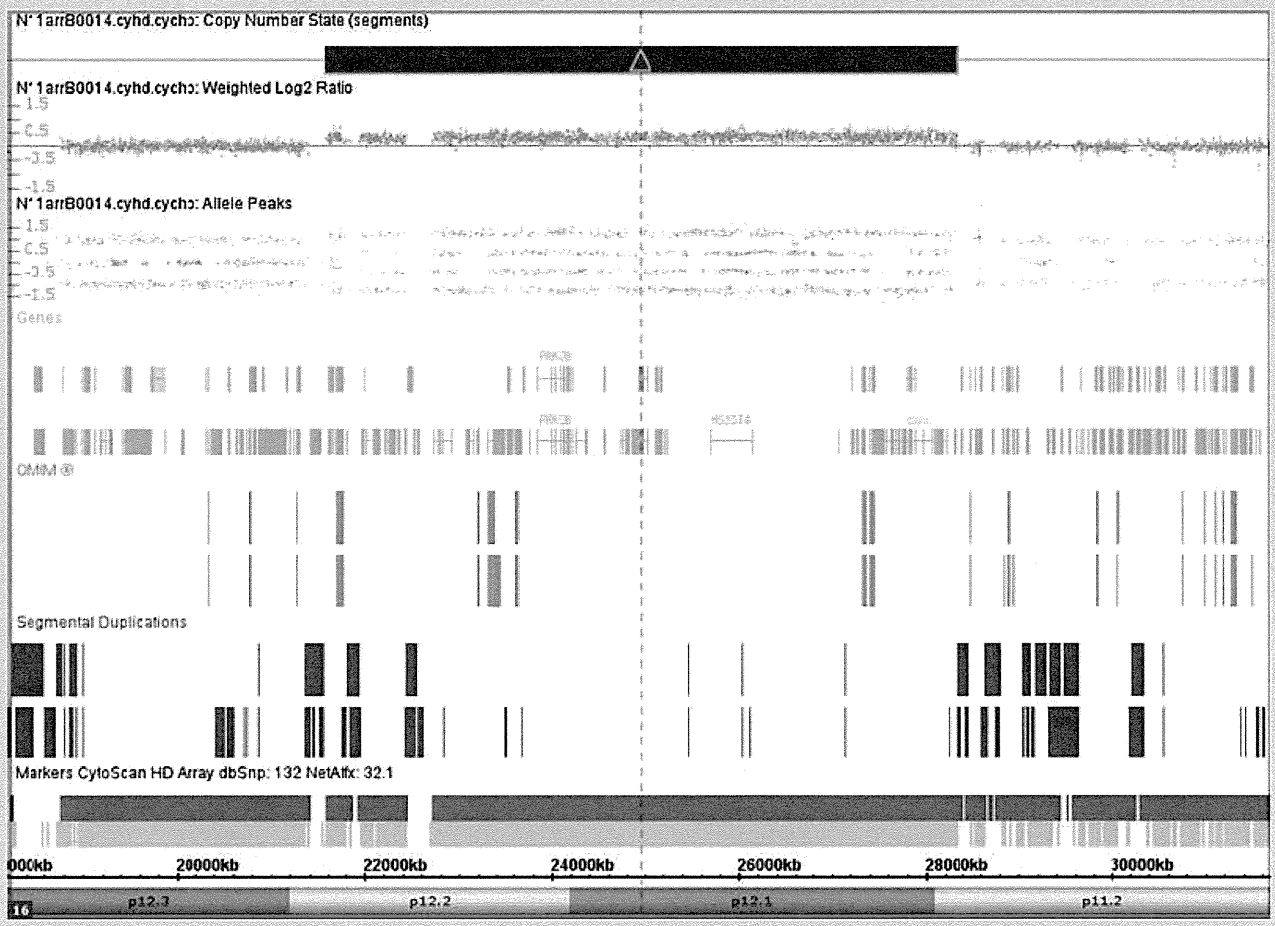

FIG. 4. Results of the SNP array analysis of Patient 2. The blue bar indicates the 16p12.2–p11.2 duplication. The duplication was 6.7 Mb. The segment contained OMIM disease genes (*OTOA*, *SCNN1G*, *SCNN1B*, *COG7*, *EARS2*, *PALB2*, *IL4R*, and *IL21R*). The duplicated region of Patient 2 was included in the deleted region of Patient 1. *SH2B1*, *ATP2A1*, and *CD19* were not included in the duplication.

including a triangular face, deep-set eyes, a large and prominent nasal bridge, and a tall, slender build. Barber et al. [2013] reported further two patients with duplication. One patient was a girl of 18 with autism, moderate ID, behavioral difficulties, dysmorphic features, and 7.71 Mb duplication. Another patient had 7.81 Mb duplication, speech delay, and obsessional behavior as a boy and, as an adult, short stature, macrocephaly and mild dysmorphism. Barber et al. [2013] suggested that the 16p12.2–p11.2 duplication syndrome is a recurrent genomic disorder with a variable phenotype.

Molecularly defined patients with the 16p12.2–p11.2 duplication syndrome are summarized in Table II. Facial features of the 16p12.2–p11.2 duplication syndrome are different among patients. Common dysmorphisms reported by Barber et al. [2013] included a depressed, broad or large nasal bridge, upslanting or narrow palpebral features, hypertelorism, and a long or tented philtrum. Patient 2 had dysmorphic features characterized by a round face, a flat nasal bridge, full cheeks, a wide philtrum, a wide mouth, a pointed chin, generalized hypertrichosis, and fetal finger pads. She

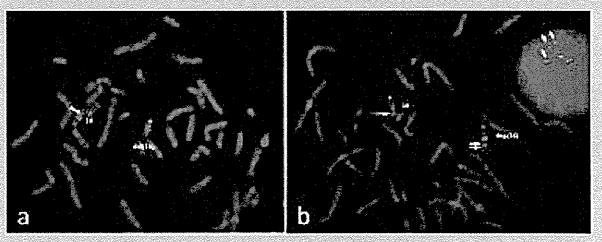

FIG. 5. A FISH analysis was performed to confirm the copy number changes. a: The red signal covering RP11-281K19 (*RBBP6*) [chr16:24,439,760–24,647,914] was deleted in patient 1. b: The red signal covering RP11-281K19 (*RBBP6*) was duplicated in Patient 2. The green signal indicates the 16q24.3 subtelomeric probe, GS-240G10. Arrows indicate RP11-281K19 (*RBBP6*).

TABLE II. Clinical Findings in Patients with 16p12.2–p11.2 Duplication Syndrome

	Finelli et al. [2004] Patient 1	Ballif et al. [2007] Subject 5	Tabet et al. [2012] Patient 2 (MZ twin)	Tabet et al. [2012] Patient 3 (MZ twin)	Barber et al. [2013] Patient 1	Barber et al. [2013] Patient 2	Present patient 1
Size of duplication [Mb]	8	5.7 Mb triplication, 1.1 Mb duplication	8.95	8.95	7.71–8.07	7.81–8.37	6.7
Age of diagnosis	25 years	10 years	17 years	18 years	15 years	45 years	9 years
General							
Gender	M	F	M	M	F	M	F
Development/neurological							
Developmental delay	+	+	+	+	+	+	+
Intellectual diability	Severe	Severe	Severe	Severe	Moderate	+	Mild
Epilepsy	+				−	−	−
EEG abnormalities	+				−		+
Autism spectrum disorders	+	+	+	+	+	−	+
Obsessive behavior	+				+	+	+
Stereotype behavior	+	+	+	+	+		
Social problems	+	+	+	+	+		−
Growth							
Height	<3rd centile	−3 to −4 SD	normal	normal	<*P*25	0.4th centile	−2.3 SD
Weight	<3rd centile	−2 SD	−2 SD	−1 SD	<*P*25		−1.2 SD
Head circumference	25th centile	−1 SD	−1 SD	−1 SD	<*P*50	Macro	1.5 SD
Facial appearance							
Round face		+	−	−			+
Triangular glabella		−	+	+			−
Promient glabella		−	+	+	+		−
Depresssed/large/broad nasal bridge	+	+	+	+	+		+
Upslanting palpebral fissures		−	+	+			−
Deep set eye		−	+	+			−
Hypertelorism	+	+	+	+			−
Nystagmus/Strabisums		+	+	+	+	+	−
Anteverted nares		+			+		−
Upper lip anomaly	+	+			−		−
Low set ears	−				−		+
Wide/large mouth	+	+					+
Full cheeks		+					+
Micro/retrognathia		−	−	−	−	+	−
Finger/hand anomalies	+	Fetal pads	+	+	−	Brachydactyly	Fetal pads
Generalized hypertrichosis							+

shared some features with other reported patients. Fetal finger pads have been found in one of the patients reported by Ballif et al. [2007]. Our results further support the evidence that ID, ASD, dysmorphic features, short stature, and anomalies of the hands and fingers are common in the 16p12.2–p11.2 duplication syndrome. However, intellectual disabilities in Patient 2 were very mild. Her communicating skill improved after 8 years old. Duplication in our patient did not include the 16p11.2 region carrying susceptibility to ASD and *SH2B1*. We suppose that differences in the proximal breakpoint may contribute to the severity of intellectual disabilities. Increased dosage of one or more genes in the duplicated region might contribute to ASD.

Delay in motor development is a constant feature of the 16p12.2–p11.2 deletion syndrome. Early development of the twins reported by Tabet et al. [2012] was normal, with walking at 15 months. The patient reported by Barber et al. [2013] walked at 13 months. Patient 2 started to walk independently at 18 months old. Delay in motor development is not considered to be a serious problem in the 16p12.2–p11.2 duplication syndrome.

Barber et al. [2013] suggested that the duplications *Polo-like kinase 1* (*PLK1*) might have an effect on neuronal development and microcephaly in human. However, one of the patients reported by them had macrocephaly and our patient also had relative macrocephaly.

We posit that the 16p12.2–p11.2 duplication is a new clinical entity with ASD due to non-allelic homologous recombination. The deletion is a MCA/ID syndrome. More patient studies will be needed to further define the syndrome.

ACKNOWLEDGMENTS

We thank for the family for their cooperation. This study was supported by the Health and Labor Research Grants in 2012 by Ministry of Health, Labor, and Welfare in Japan.

REFERENCES

Bachmann-Gagescu R, Mefford HC, Cowan C, Glew GM, Hing AV, Wallace S, Bader PI, Hamati A, Reitnauer PJ, Smith R, Stockton DW, Muhle H, Helbig I, Eichler EE, Ballif BC, Rosenfeld J, Tsuchiya KD. 2010. Recurrent 200-kb deletions of 16p11.2 that include the SH2B1 gene are associated with developmental delay and obesity. Genet Med 12:641–647.

Ballif BC, Hornor SA, Jenkins E, Madan-Khetarpal S, Surti U, Jackson KE, Asamoah A, Brock PL, Gowans GC, Conway RL, Graham JM, Jr. Medne L, Zackai EH, Shaikh TH, Geoghegan J, Selzer RR, Eis PS, Bejjani BA, Shaffer LG. 2007. Discovery of a previously unrecognized microdeletion syndrome of 16p11.2-p12.2. Nat Genet 39:1071–1073.

Barber JC, Hall V, Maloney VK, Huang S, Roberts AM, Brady AF, Foulds N, Bewes B, Volleth M, Liehr T, Mehnert K, Bateman M, White H. 2013. 16p11.2-p12.2 duplication syndrome; a genomic condition differentiated from euchromatic variation of 16p11.2. Eur J Hum Genet 21:182–189.

Battaglia A, Novelli A, Bernardini L, Igliozzi R, Parrini B. 2009. Further characterization of the new microdeletion syndrome of 16p11.2-p12.2. Am J Med Genet A 149A:1200–1204.

Bochukova EG, Huang N, Keogh J, Henning E, Purmann C, Blaszczyk K, Saeed S, Hamilton-Shield J, Clayton-Smith J, O'Rahilly S, Hurles ME, Farooqi IS. 2010. Large, rare chromosomal deletions associated with severe early-onset obesity. Nature 463:666–670.

Engelen JJ, de Die-Smulders CE, Dirckx R, Verhoeven WM, Tuinier S, Curfs LM, Hamers AJ. 2002. Duplication of chromosome region (16)(p11.2-->p12.1) in a mother and daughter with mild mental retardation. Am J Med Genet 109:149–153.

Fernandez BA, Roberts W, Chung B, Weksberg R, Meyn S, Szatmari P, Joseph-George AM, Mackay S, Whitten K, Noble B, Vardy C, Crosbie V, Luscombe S, Tucker E, Turner L, Marshall CR, Scherer SW. 2010. Phenotypic spectrum associated with de novo and inherited deletions and duplications at 16p11.2 in individuals ascertained for diagnosis of autism spectrum disorder. J Med Genet 47:195–203.

Finelli P, Natacci F, Bonati MT, Gottardi G, Engelen JJ, de Die-Smulders CE, Sala M, Giardino D, Larizza L. 2004. FISH characterisation of an identical (16)(p11.2p12.2) tandem duplication in two unrelated patients with autistic behaviour. J Med Genet 41:e90.

Girirajan S, Rosenfeld JA, Cooper GM, Antonacci F, Siswara P, Itsara A, Vives L, Walsh T, McCarthy SE, Baker C, Mefford HC, Kidd JM, Browning SR, Browning BL, Dickel DE, Levy DL, Ballif BC, Platky K, Farber DM, Gowans GC, Wetherbee JJ, Asamoah A, Weaver DD, Mark PR, Dickerson J, Garg BP, Ellingwood SA, Smith R, Banks VC, Smith W, McDonald MT, Hoo JJ, French BN, Hudson C, Johnson JP, Ozmore JR, Moeschler JB, Surti U, Escobar LF, El-Khechen D, Gorski JL, Kussmann J, Salbert B, Lacassie Y, Biser A, McDonald-McGinn DM, Zackai EH, Deardorff MA, Shaikh TH, Haan E, Friend KL, Fichera M, Romano C, Gécz J, DeLisi LE, Sebat J, King MC, Shaffer LG, Eichler EE. 2010. A recurrent 16p12.1 microdeletion supports a two-hit model for severe developmental delay. Nat Genet 42:203–209.

Hempel M, Rivera Brugués N, Wagenstaller J, Lederer G, Weitensteiner A, Seidel H, Meitinger T, Strom TM. 2009. Microdeletion syndrome 16p11.2-p12.2: Clinical and molecular characterization. Am J Med Genet A 149A:2106–2112.

Hernando C, Plaja A, Rigola MA, Pérez MM, Vendrell T, Egocue J, Fuster C. 2002. Comparative genomic hybridisation shows a partial de novo deletion 16p11.2 in a neonate with multiple congenital malformations. J Med Genet 39:e24.

Kumar RA, KaraMohamed S, Sudi J, Conrad DF, Brune C, Badner JA, Gilliam TC, Nowak NJ, Cook EH, Jr. Dobyns WB, Christian SL. 2008. Recurrent 16p11.2 microdeletions in autism. Hum Mol Genet 17:628–638.

Marshall CR, Noor A, Vincent JB, Lionel AC, Feuk L, Skaug J, Shago M, Moessner R, Pinto D, Ren Y, Thiruvahindrapduram B, Fiebig A, Schreiber S, Friedman J, Ketelaars CE, Vos YJ, Ficicioglu C, Kirkpatrick S, Nicolson R, Sloman L, Summers A, Gibbons CA, Teebi A, Chitayat D, Weksberg R, Thompson A, Vardy C, Crosbie V, Luscombe S, Baatjes R, Zwaigenbaum L, Roberts W, Fernandez B, Szatmari P, Scherer SW. 2008. Structural variation of chromosomes in autism spectrum disorder. Am J Hum Genet 82:477–488.

Tabet AC, Pilorge M, Delorme R, Amsellem F, Pinard JM, Leboyer M, Verloes A, Benzacken B, Betancur C. 2012. Autism multiplex family with 16p11.2p12.2 microduplication syndrome in monozygotic twins and distal 16p11.2 deletion in their brother. Eur J Hum Genet 20:540–546.

Weiss LA, Shen Y, Korn JM, Arking DE, Miller DT, Fossdal R, Saemundsen E, Stefansson H, Ferreira MA, Green T, Platt OS, Ruderfer DM, Walsh CA, Altshuler D, Chakravarti A, Tanzi RE, Stefansson K, Santangelo SL, Gusella JF, Sklar P, Wu BL, Daly MJ, Autism Consortium. 2008. Association between microdeletion and microduplication at 16p11.2 and autism. N Engl J Med 358:667–675.

CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

109 kb Deletion of Chromosome 4p16.3 in a Patient With Mild Phenotype of Wolf–Hirschhorn Syndrome

Nobuhiko Okamoto,[1]* Kazumi Ohmachi,[1] Shino Shimada,[2,3] Keiko Shimojima,[2] and Toshiyuki Yamamoto[2]

[1]Department of Medical Genetics, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan

[2]Tokyo Women's Medical University Institute for Integrated Medical Sciences, Tokyo, Japan

[3]Department of Pediatrics, Tokyo Women's Medical University, Tokyo, Japan

Manuscript Received: 13 August 2012; Manuscript Accepted: 26 January 2013

Wolf–Hirschhorn syndrome (WHS) is a contiguous gene deletion syndrome associated with growth retardation, developmental disabilities, epileptic seizures, and distinct facial features resulting from a deletion of the short arm of chromosome 4. The Wolf–Hirschhorn Syndrome Critical Region WHSCR2 includes the *LETM1* gene and 5′ end of the *WHSC1* gene. A haploinsufficiency of *WHSC1* is thought to be responsible for a number of WHS characteristics. We report on a 2-year-old male with severe growth retardation, microcephaly and a characteristic facial appearance. He had no internal anomalies and his developmental milestones were mildly delayed. An array-CGH analysis revealed loss of genomic copy numbers in the region 4p16.3, which included *FGFR3*, *LETM1*, and *WHSC1*. The size of the deletion was only 109 kb. The deletion included the important genes in WHSCR2. We suspect that haploinsufficiency of *WHSC1* is the most probable cause of the growth deficiency, microcephaly, and characteristic facial features in WHS.

Key words: *WHSC1*; *LETM1*; Wolf–Hirschhorn syndrome; WHSCR1; WHSCR2

How to Cite this Article:
Okamoto N, Ohmachi K, Shimada S, Shimojima K, Yamamoto T. 2013. 109 kb deletion of chromosome 4p16.3 in a patient with mild phenotype of Wolf–Hirschhorn syndrome.
Am J Med Genet Part A 161A:1465–1469.

INTRODUCTION

Wolf–Hirschhorn syndrome (WHS) is a contiguous gene deletion syndrome associated with growth retardation, developmental disabilities, epileptic seizures, and distinct facial features resulting from a deletion of the short arm of chromosome 4. Two critical regions for WHS have been mapped to a 200 kb area about 1.9 Mb from the 4p telomere. Wolf–Hirschhorn Syndrome Critical Region 1 (WHSCR1) is a 165 kb stretch proximal to the *FGFR3* and *LETM1* genes [Wright et al., 1997]. Zollino et al. [2003] and Rodríguez et al. [2005] established a new critical region, WHSCR2. WHSCR2 is distal to WHSCR1 and directly adjacent to it. WHSCR2 includes the *LETM1* gene and 5′ end of the *WHSC1* gene. But the distal boundary of WHSCR2 is not well defined. South et al. [2008] and Engbers et al. [2009] reported patients with deletions distal to both critical regions. *WHSC1* is a member of a nuclear receptor binding SET domain (NSD) protein that forms a family of three histone–methyltransferase proteins. A haploinsufficiency of *WHSC1* is believed to be responsible for a number of WHS characteristics.

We report on a patient with severe growth retardation, microcephaly, and a characteristic facial appearance. An array–CGH analysis revealed loss of genomic copy numbers in the region 4p16.3, which included *FGFR3*, *LETM1*, and 5′ end of *WHSC1*. The size of the deletion was only 109 kb. Haploinsufficiency of the genes and clinical features of the patient are discussed.

CLINICAL REPORT

The 2-year-old male propositus was the second-born child of a 26-year-old mother and a 30-year-old father, both healthy and nonconsanguineous. Fetal echogram revealed intrauterine growth retardation. He was born at 39 weeks of gestation by induced delivery.

Grant sponsor: Ministry of Health, Labor and Welfare in Japan.
*Correspondence to:
Dr. Nobuhiko Okamoto, Department of Medical Genetics, Osaka Medical Center and Research Institute for Maternal and Child Health, 840 Murodo-cho, Izumi, Osaka 594-1101, Japan.
E-mail: okamoto@osaka.email.ne.jp
Article first published online in Wiley Online Library (wileyonlinelibrary.com): 1 May 2013
DOI 10.1002/ajmg.a.35910

His birth weight was 1,580 g (−3.4 SD), body length 40.5 cm (−4.1 SD), and OFC 29.5 cm (−2.2 SD).

The patient fed poorly and his physical growth was severely retarded from early infancy. However, his developmental milestones were mildly delayed. He was able to roll over at 10 months of age, and sat alone at 12 months of age. He started to walk independently at 14 months of age. Generalized hypotonia was not present.

Physical examination identified dysmorphic features, including microcephaly, triangular face, apparent hypertelorism, prominent glabella, high nasal bridge, bilateral low set ears, downslanting palpebral fissures, short philtrum, high palate, downturned mouth, and micrognathia (Fig. 1). Hearing and visual acuity were normal. Abdominal exam revealed no abnormalities. External genitalia were normal. His weight was 6.4 kg (−4.4 SD), length was 75.7 cm (−4.4 SD) and head circumference was 42 cm (−4.1 SD). His development quotient (DQ) was 71 at 2 years and 6 months of age by the Japanese standard method. His DQ for three subscales, posture-motor, cognition-adaptation and language-social, were 123, 68, and 65, respectively. His gross motor development was rather advanced. He showed hyperactivity and aggressive behavior. He could speak several words and short phrases. He understood simple sentences. Gradually, his food intake improved. He was free from febrile convulsions and epileptic seizures.

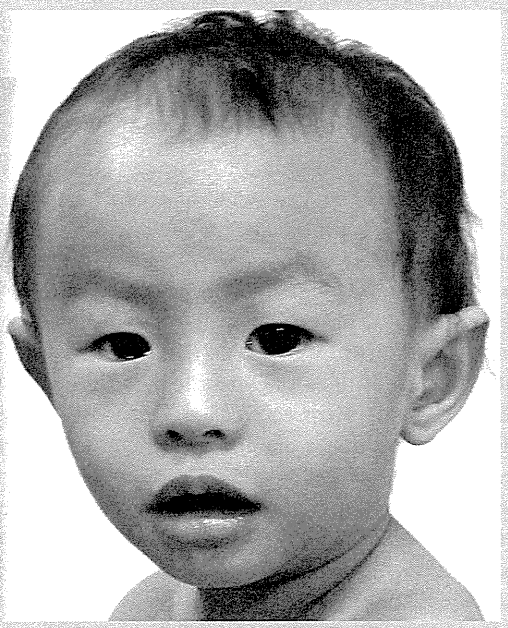

FIG. 1. Facial appearance of the patient at 2 years of age. Dysmorphic features included microcephaly, triangular face, apparent hypertelorism, prominent glabella, high nasal bridge, bilateral low-set ears, downslanting palpebral fissures, short philtrum, high palate, down turned mouth, and micrognathia. Permission for presentation has been obtained from his parents.

Results of neuroradiological examinations including brain CT and MRI were normal. Cardiac and abdominal echograms were normal. EEG showed no epileptic discharges. Routine laboratory tests were normal. His karyotype by G-banded analysis was 46,XY. Array-CGH analyses were performed to reveal submicroscopic chromosomal aberrations.

MATERIALS AND METHODS

After obtaining informed consent, peripheral blood samples were drawn from the patient and his parents. Genomic DNA was extracted using the QIAquick DNA extraction kit (QIAgen, Valencia, CA).

Based on the hypothesis that the patient might have submicroscopic chromosomal aberrations, an array-CGH analysis was performed using the SurePrint G3 Hmn CGH + SNP 180K Mircroarray Kit (Agilent Technologies, Santa Clara, CA).

Metaphase nuclei were prepared from peripheral blood lymphocytes by standard methods and used for FISH with human BAC clones selected from the UCSC genome browser (http://www.genome.ucsc.edu) as described elsewhere [Shimojima et al., 2009].

RESULTS

By array-CGH analysis, loss of genomic copy numbers was identified in the region 4p16.3, which included *FGFR3*, *LETM1*, and 5′ end of *WHSC1* (Figs. 2 and 3). FISH analyses confirmed the deletion (Fig. 4). No other significant copy number changes or long contiguous stretches of homozygosity were detected. The karyotype of the patient was arr 4p16.3 (1,792,001–1,900,840) × 1 dn. The size of the interstitial deletion was 109 kb. FISH results for the parents were normal suggesting a de novo deletion (data not shown). The parents of the patient were studied by FISH only.

DISCUSSION

A patient with severe growth retardation, microcephaly and characteristic facial features had a submicroscopic deletion of 4p16.3. Although he had the core WHS features, they were less marked. His gross motor function was beyond average. But he demonstrated mild delay in cognitive and language skills. He did not have internal anomalies including cardiac malformations and renal hypoplasia. He was free from seizures. No structural CNS defects were observed.

The deletion involved only three genes, *FGFR3*, *LETM1*, and 5′ end of *WHSC1*. Although the distal boundary of WHSCR2 is not well defined, the segment was almost compatible with WHSCR2. As the 3′ end of *WHSC1* is preserved, *WHSC1* may have retained function due to partial deletion of a gene with multiple apparent isoforms. Major transcription isoforms of *WHSC1* seem to use 5′ end of the gene. Deletion of the 5′ end of the gene will affect its function the reported deletion results in the stated haploinsufficiency of this gene.

FGFR3-related skeletal disorders are caused by gain of function mutations. Fgfr3−/− mice show severe skeletal anomalies and inner ear defects, however, Fgfr3+/− mice show no phenotypic abnormalities [Colvin et al., 1996; Deng et al., 1996]. The hap-

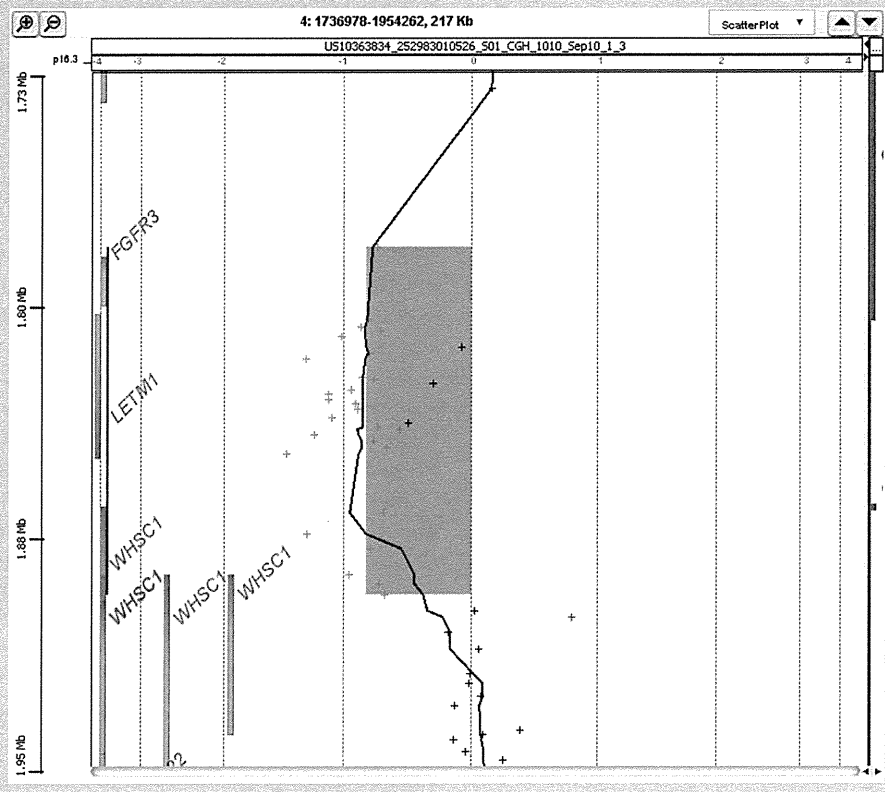

FIG. 2. Array-CGH revealed loss of genomic copy numbers in the region 4p16.3, which included the *FGFR3*, *LETM1*, and 5′ end of *WHSC1*. Blue rectangle indicates the region of 109 kb copy loss. Major transcript variants of *WHSC1* are shown by four bars. The deletion involved 5′ part of the major *WHSC1* transcript variants.

loinsufficiency of *FGFR3* may not have affected the patient's clinical features.

LETM1 is deleted in almost all patients with WHS and has been suggested as a candidate gene responsible for seizures. Schlickum et al. [2004] showed that *LETM1* is evolutionarily conserved and exhibits homology to a putative yeast protein involved in mitochondrial morphology. They suggested that some neuromuscular features of WHS may be caused by mitochondrial dysfunction. Dimmer et al. [2008] found that human *LETM1* is located in the inner mitochondrial membrane, exposed to the matrix and oligomerized in higher molecular weight complexes of unknown composition. They reported that down-regulation of *LETM1* expression did not disrupt these complexes, but led to fragmentation of the mitochondrial network and "necrosis-like" death. Fibroblasts from a WHS patient displayed reduced *LETM1* mRNA and protein levels, but mitochondrial morphology was unaffected. McQuibban et al. [2010] identified the Drosophila ortholog of *LETM1* and named the gene *DmLETM1*. They demonstrated that the product of *DmLETM1* function as a mitochondrial osmoregulator through its K(+)/H(+) exchange activity. Conditional inactivation of *DmLETM1* results in roughening of the adult eye, mitochondrial swelling and developmental lethality in third-instar larvae, possibly the result of deregulated mitophagy. Neuronal specific down-regulation of DmLETM1 results in an impairment of locomotor behavior in the fly and reduced synaptic neurotransmitter release.

South et al. [2007] reported two patients with terminal microdeletions in 4p16.3 that exclude the WHS critical regions. Both patients showed significant postnatal growth delay, mild developmental delays, and feeding difficulties. Their facial features were not typical for WHS. A portion of *LETM1* was deleted in the patient with seizures. Their results supported the hypothesis that a gene in WHSCR2, *LETM1*, plays a direct role in seizure development. Maas et al. [2008] reported that a patient with the 1.4 Mb terminal 4p deletion without the *LETM1* deletion did present with seizures. They suggested that another gene in the terminal region may cause the epilepsy. Battaglia et al. [2009] reported that epilepsy occurred in 81 patients (93%) among 87 WHS patients within the first 3 years of life. Status epilepticus occurred in 50% of the patients under 3 years of age. Although our patient is still 2 years and 6 months old, he is free from epileptic seizures. Beside the extent of the 4p deletion, seizures are a prognostic factor for degree of intellectual disability [Zollino et al., 2008]. We suppose that *LETM1* haploinsufficiency may not always cause epileptic seizures. The mild degree of intellectual disability in our patient may come from absence of seizures. We are planning further clinical observation with repeated EEG studies.

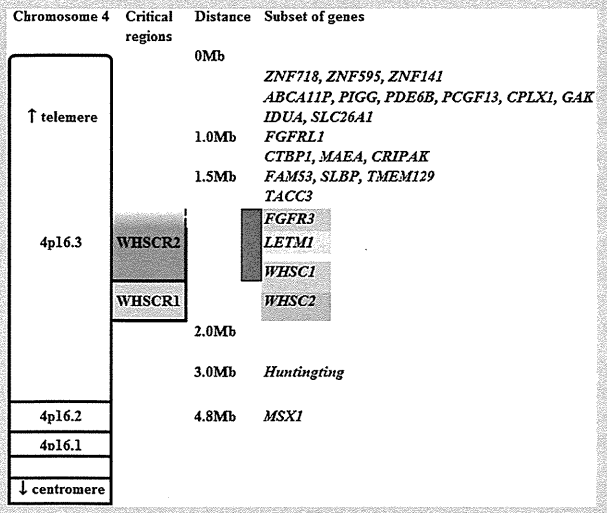

FIG. 3. Schematic presentation of genomic map of the 4p terminal region. The red bar indicates the deletion. The distal boundary of WHSCR2 is not well defined. It is represented by fading color. Subset of genes distal to *FGFR3* and proximal to *WHSC2* was preserved.

WHSC1 is a member of a family of methyltransferase proteins. The nuclear receptor binding SET domain (NSD) protein is a family of three HMTases, NSD1, NSD2/MMSET/WHSC1, and NSD3/WHSC1L1. NSD proteins are critical in maintaining chromatin integrity. Haploinsufficiency of *NSD1* is the major cause of Sotos syndrome [Kurotaki et al., 2002]. *NSD3/WHSC1L1* closely resembles *NSD2*. No genetic disorders are known to be associated with *NSD3/WHSC1L1*.

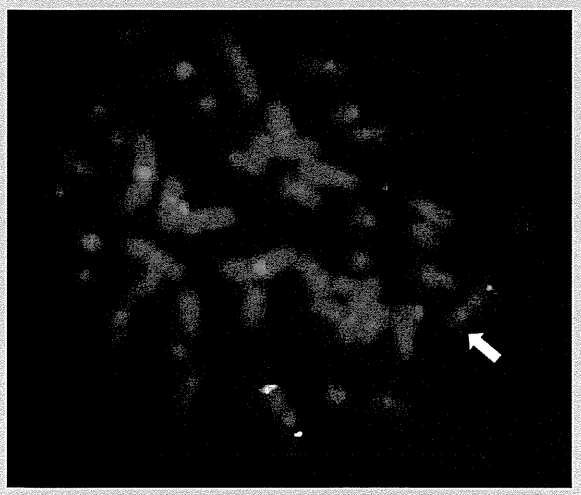

FIG. 4. FISH analyses confirmed the deletion at 4p16.3 (arrow). The deletion was de novo. FISH probe spectrum orange: RP11-28I12 (4q35) spectrum green: CTD-2269L21 (4p16.3).

Nimura et al. [2009] found that mouse Whsc1 governed H3K36me3 along euchromatin by associating with the cell-type-specific transcription factors *Sall1*, *Sall4*, and *Nanog* in embryonic stem cells, and *Nkx2–5* in embryonic hearts, regulating the expression of their target genes. *Whsc1*-deficient mice showed growth retardation and various congenital anomalies, including congenital cardiovascular anomalies. The effects of *Whsc1* haploinsufficiency were increased in *Nkx2–5* heterozygous mutant hearts, indicating their functional link. Nimura et al. [2009] proposed that *WHSC1* functions together with other genetic factors to prevent the inappropriate transcription that can lead to various pathophysiologies.

Hajdu et al. [2011] reported that the WHSC1 protein is a member of the DNA damage response pathway. WHSC1 localizes to sites of DNA damage and replication stress and is required for resistance to many DNA-damaging and replication stress-inducing agents. They proposed that WHSC1 has important roles in the DNA damage and DNA replication stress response. The developmental and neurological impairment in WHS may be explained by the defect in DNA damage and replication.

Van Buggenhout et al. [2004] identified six mild WHS patients with small 4p deletions using a micro-array CGH analysis. WHSC1 was the only common deleted gene. They concluded that WHSC1 haploinsufficiency is essential to the development of the typical facial appearance. Engbers et al. [2009] reported a 1.9-year-old girl with developmental delay and several facial characteristics reminiscent of WHS, who carried a terminal 4p16.3 deletion. The *FGFRL1* gene was deleted, but *WHSC1* was preserved. The patient had no microcephaly and only mild intellectual disability. Her body length was 81 cm (5th centile for age). They suggested that *FGFRL1* represents a plausible candidate gene for part of the facial characteristics of WHS. Izumi et al. [2010] reported a patient with a 1.3 Mb interstitial deletion of 4p16.3 involving *WHSC1* and suggested that *WHSC1* haploinsufficiency contributed to the pathogenesis of severe developmental delay.

Luo et al. [2011] reported a 54 kb deletion of 4p16.3 that includes *LETM1*. The patient exhibited no facial features of WHS. She was referred for testing at 1 year of age, presenting with microtia, renal agenesis, Duane anomaly and a congenital heart defect. These data suggest that loss of *LETM1* is not responsible for the characteristic facial features in WHS and other candidate genes in the critical region may be involved.

We suspect that haploinsufficiency of *WHSC1* is the most probable cause of severe growth deficiency, microcephaly and characteristic facial features in our patient. However, his DQ was 71 at 2 years and 6 months of age. This indicates that *WHSC1* haploinsufficiency is not enough to cause severe intellectual disability. Our patient showed less marked craniofacial features of WHS. We suggest that *WHCS1* and other distally located genes have cumulative effect on the severe intellectual disability and typical craniofacial features in WHS.

In conclusion, we reported on a patient with a 109 kb deletion in 4p16.3 with a mild phenotype of WHS. The deletion was compatible with WHSCR2. This patient is a good model to understand the role of *WHSC1*. We suppose that single gene disorder of *WHSC1* might have similar conditions.

ACKNOWLEDGMENTS

We thank for the family for their cooperation. This study was supported by the Health and Labor Research Grants in 2012 by Ministry of Health, Labor and Welfare in Japan.

REFERENCES

Battaglia A, Filippi T, South ST, Carey JC. 2009. Spectrum of epilepsy and electroencephalogram patterns in Wolf–Hirschhorn syndrome: Experience with 87 patients. Dev Med Child Neurol 51:373–380.

Colvin JS, Bohne BA, Harding GW, McEwen DG, Ornitz DM. 1996. Skeletal overgrowth and deafness in mice lacking fibroblast growth factor receptor 3. Nat Genet 12:390–397.

Deng C, Wynshaw-Boris A, Zhou F, Kuo A, Leder P. 1996. Fibroblast growth factor receptor 3 is a negative regulator of bone growth. Cell 84:911–921.

Dimmer KS, Navoni F, Casarin A, Trevisson E, Endele S, Winterpacht A, Salviati L, Scorrano L. 2008. LETM1, deleted in Wolf–Hirschhorn syndrome is required for normal mitochondrial morphology and cellular viability. Hum Mol Genet 17:201–214.

Engbers H, van der Smagt JJ, van 't Slot R, Vermeesch JR, Hochstenbach R, Poot M. 2009. Wolf–Hirschhorn syndrome facial dysmorphic features in a patient with a terminal 4p16.3 deletion telomeric to the WHSCR and WHSCR 2 regions. Eur J Hum Genet 17:129–132.

Hajdu I, Ciccia A, Lewis SM, Elledge SJ. 2011. Wolf–Hirschhorn syndrome candidate 1 is involved in the cellular response to DNA damage. Proc Natl Acad Sci USA 108:13130–13134.

Izumi K, Okuno H, Maeyama K, Sato S, Yamamoto T, Torii C, Kosaki R, Takahashi T, Kosaki K. 2010. Interstitial microdeletion of 4p16.3: Contribution of WHSC1 haploinsufficiency to the pathogenesis of developmental delay in Wolf–Hirschhorn syndrome. Am J Med Genet Part A 152A:1028–1032.

Kurotaki N, Imaizumi K, Harada N, Masuno M, Kondoh T, Nagai T, Ohashi H, Naritomi K, Tsukahara M, Makita Y, Sugimoto T, Sonoda T, Hasegawa T, Chinen Y, Tomita Ha HA, Kinoshita A, Mizuguchi T, Yoshiura Ki K, Ohta T, Kishino T, Fukushima Y, Niikawa N, Matsumoto N. 2002. Haploinsufficiency of NSD1 causes Sotos syndrome. Nat Genet 30:365–366.

Luo Y, Hermetz KE, Jackson JM, Mulle JG, Dodd A, Tsuchiya KD, Ballif BC, Shaffer LG, Cody JD, Ledbetter DH, Martin CL, Rudd MK. 2011. Diverse mutational mechanisms cause pathogenic subtelomeric rearrangements. Hum Mol Genet 20:3769–3778.

Maas NM, Van Buggenhout G, Hannes F, Thienpont B, Sanlaville D, Kok K, Midro A, Andrieux J, Anderlid BM, Schoumans J, Hordijk R, Devriendt K, Fryns JP, Vermeesch JR. 2008. Genotype–phenotype correlation in 21 patients with Wolf–Hirschhorn syndrome using high resolution array comparative genome hybridisation (CGH). J Med Genet 45:71–80.

McQuibban AG, Joza N, Megighian A, Scorzeto M, Zanini D, Reipert S, Richter C, Schweyen RJ, Nowikovsky K. 2010. A Drosophila mutant of LETM1, a candidate gene for seizures in Wolf–Hirschhorn syndrome. Hum Mol Genet 19:987–1000.

Nimura K, Ura K, Shiratori H, Ikawa M, Okabe M, Schwartz RJ, Kaneda Y. 2009. A histone H3 lysine 36 trimethyltransferase links *Nkx 2–5* to Wolf–Hirschhorn syndrome. Nature 460:287–291.

Rodríguez L, Zollino M, Climent S, Mansilla E, López-Grondona F, Martínez-Fernández ML, Murdolo M, Martínez-Frías ML. 2005. The new Wolf–Hirschhorn syndrome critical region (WHSCR-2): A description of a second case. Am J Med Genet Part A 136A:175–178.

Schlickum S, Moghekar A, Simpson JC, Steglich C, O'Brien RJ, Winterpacht A, Endele SU. 2004. LETM1, a gene deleted in Wolf–Hirschhorn syndrome, encodes an evolutionarily conserved mitochondrial protein. Genomics 83:254–261.

Shimojima K, Páez MT, Kurosawa K, Yamamoto T. 2009. Proximal interstitial 1p36 deletion syndrome: The most proximal 3.5-Mb microdeletion identified on a dysmorphic and mentally retarded patient with inv(3)(p14.1q26.2). Brain Dev 31:629–633.

South ST, Bleyl SB, Carey JC. 2007. Two unique patients with novel microdeletions in 4p16.3 that exclude the WHS critical regions: Implications for critical region designation. Am J Med Genet Part A 143A: 2137–2142.

South ST, Hannes F, Fisch GS, Vermeesch JR, Zollino M. 2008. Pathogenic significance of deletions distal to the currently described Wolf–Hirschhorn syndrome critical regions on 4p16.3. Am J Med Genet Part C Semin Med Genet 148C:270–274.

Van Buggenhout G, Melotte C, Dutta B, Froyen G, Van Hummelen P, Marynen P, Matthijs G, de Ravel T, Devriendt K, Fryns JP, Vermeesch JR. 2004. Mild Wolf–Hirschhorn syndrome: Micro-array CGH analysis of atypical 4p16.3 deletions enables refinement of the genotype–phenotype map. J Med Genet 41:691–698.

Wright TJ, Ricke DO, Denison K, Abmayr S, Cotter PD, Hirschhorn K, Keinänen M, McDonald-McGinn D, Somer M, Spinner N, Yang-Feng T, Zackai E, Altherr MR. 1997. A transcript map of the newly defined 165 kb Wolf–Hirschhorn syndrome critical region. Hum Mol Genet 6:317–324.

Zollino M, Lecce R, Fischetto R, Murdolo M, Faravelli F, Selicorni A, Buttè C, Memo L, Capovilla G, Neri G. 2003. Mapping the Wolf–Hirschhorn syndrome phenotype outside the currently accepted WHS critical region and defining a new critical region, WHSCR-2. Am J Hum Genet 72:590–597.

Zollino M, Murdolo M, Marangi G, Pecile V, Galasso C, Mazzanti L, Neri G. 2008. On the nosology and pathogenesis of Wolf–Hirschhorn syndrome: Genotype–phenotype correlation analysis of 80 patients and literature review. Am J Med Genet Part C Semin Med Genet 148C:257–269.