

**Microarray and FISH-based Genotype-Phenotype Analysis of 22 Japanese Patients
with Wolf-Hirschhorn Syndrome**

**Kenji Shimizu,^{1,2} Keiko Wakui,¹ Tomoki Kosho,^{1*} Nobuhiko Okamoto,³ Seiji
Mizuno,⁴ Kazuya Itomi,⁵ Shigeto Hattori,⁶ Kimio Nishio,⁷ Osamu Samura,⁸ Yoshiyuki
Kobayashi,⁹ Yuko Kako,¹⁰ Takashi Arai,¹¹ Tsutomu Oishi,¹¹ Hiroshi Kawame,¹² Yoko
Narumi,¹ Hirofumi Ohashi,² and Yoshimitsu Fukushima¹**

¹Department of Medical Genetics, Shinshu University School of Medicine, Matsumoto,
Japan

²Division of Medical Genetics, Saitama Children's Medical Center, Saitama, Japan

³Department of Medical Genetics, Osaka Medical Center and Research Institute for
Maternal and Child Health, Osaka, Japan

⁴Department of Pediatrics, Central Hospital, Aichi Human Service Center, Kasugai, Aichi,
Japan

⁵Department of Pediatric Neurology, Aichi Children's Health and Medical Center, Obu,
Aichi, Japan

⁶Department of Pediatrics, Gunma University Hospital, Maebashi, Japan.

⁷Department of Neonatology, Seirei-Hamamatsu General Hospital, Hamamatsu, Shizuoka,
Japan

⁸Department of Obstetrics and Gynecology, NHO Kure Medical Center and Chugoku
Cancer Center, Kure, Japan

⁹Department of Pediatrics, Hiroshima University Hospital, Hiroshima, Japan.

¹⁰Department of Pediatrics, Showa University School of Medicine, Tokyo, Japan

¹¹Division of Clinical Laboratory, Saitama Children's Medical Center, Saitama, Japan

¹²Department of Genetic Counseling, Graduate School of Humanities and Science,

Ochanomizu University, Tokyo, Japan

Running Heads:

Shimizu et al.

Wolf-Hirschhorn syndrome in Japan

*Correspondence to:

Tomoki Kosho, MD, Department of Medical Genetics, Shinshu University School of
Medicine, 3-1-1 Asahi, Matsumoto 390-8621, Japan.

Phone: +81-263-37-2618; Fax: +81-263-37-2619;

E-mail: ktomoki@shinshu-u.ac.jp

ABSTRACT

Wolf-Hirschhorn syndrome (WHS) is a contiguous gene deletion syndrome of the distal 4p chromosome, characterized by craniofacial features, growth impairment, intellectual disability, and seizures. Although genotype-phenotype correlation studies have previously been published, several important issues remain to be elucidated including seizure severity. We present detailed clinical and molecular-cytogenetic findings from a microarray and fluorescence *in situ* hybridization (FISH)-based genotype-phenotype analysis of 22 Japanese WHS patients, the first large non-Western series. 4p deletions were terminal in 20 patients and interstitial in two, with deletion sizes ranging from 2.06–29.42 Mb. The new Wolf-Hirschhorn syndrome critical region (WHSCR2) was deleted in all cases, and duplication of other chromosomal regions occurred in four. Complex mosaicism was identified in two cases: two different 4p terminal deletions, a simple 4p terminal deletion and an unbalanced translocation with the same 4p breakpoint. Seizures began in infancy in 33% (2/6) of cases with small (<6 Mb) deletions and in 86% (12/14) of cases with larger deletions (>6 Mb). Status epilepticus occurred in 17% (1/6) with small deletions and in 87% (13/15) with larger deletions. Renal hypoplasia or dysplasia and structural ocular anomalies were more prevalent in those with larger deletions. A new susceptible region for seizure occurrence is suggested between 0.76–1.3 Mb from 4pter, encompassing *CTBPI* and *CPLX1*, and distal to the previously-supposed candidate gene *LETM1*. The usefulness of bromide therapy for seizures and additional clinical features including hypercholesterolemia are also described.

KEY WORDS:

Wolf-Hirschhorn syndrome; 4p deletion; microarray analysis; fluorescence *in situ*

hybridization (FISH); mosaicism; genotype-phenotype correlation; seizures

INTRODUCTION

Wolf-Hirschhorn syndrome (WHS, OMIM#194190), first described independently by Hirschhorn et al. [1965] and Wolf et al. [1965], is a contiguous gene deletion syndrome of the distal 4p chromosome characterized by a distinctive facial appearance, pre- and postnatal growth impairment, intellectual disability, and seizures [Battaglia et al., 2008]. In recent years, chromosomal microarray-based analysis has enabled us to identify WHS patients harboring well-defined variable-sized 4p deletions with or without additional duplications of other chromosomal regions as a result of unbalanced derivatives determined by concurrent metaphase fluorescence *in situ* hybridization (FISH) analysis [South et al., 2008c; Zollino et al., 2008].

Detailed genotype-phenotype correlation studies have also been performed using FISH analysis [Battaglia et al., 1999; Zollino et al., 2000] and chromosomal microarray analysis [Battaglia et al., 2008; Maas et al., 2008; South et al., 2008c; Zollino et al., 2008]. Clinical severity generally correlates with deletion sizes, although co-existing duplicated chromosomal regions, sequence variation of the gene(s) in the non-deleted 4p region, and other genetic backgrounds can all contribute to phenotypic variation [South et al., 2008c; Zollino et al., 2008]. Seizures represent a major clinical challenge in patients with WHS [Battaglia et al., 2009]. A review by Zollino et al. [2008] showed a high prevalence of seizures in patients with WHS regardless of the deletion sizes: 96% in those with <3.5 Mb deletions, 80% in those with 5–18 Mb deletions, and 90% in those with >22 Mb deletions. However, it remains unclear whether the severity of seizures in WHS patients might correlate with deletion sizes.

A WHS critical region, responsible for cardinal WHS features such as distinctive faces, growth/developmental delay, and seizures, was initially mapped to a 165 kb interval (WHSCR1) involving the entire *WHSC2* gene and the proximal part of *WHSC1* [Wright et

al., 1997]. Additional reports of WHS patients with deleted regions distal to WHSCR1 suggested a new critical region (WHSCR2) involving the distal part of *WHSC1* and the entire *LETMI* gene, and these two genes have been considered the molecular hallmark of WHS [Zollino et al., 2003; Rodriguez et al., 2005]. Indeed, *WHSC1* is hypothesized as the gene that contributes to the main WHS phenotype of developmental delay and characteristic facial features [Nimura et al., 2009; Izumi et al., 2010]. *LETMI*, encoding a mitochondrial $\text{Ca}^{2+}/\text{H}^{+}$ antiporter [Jiang et al., 2009], is thought to be the major candidate gene for seizures in patients with WHS [Rauch et al., 2001; South et al., 2007], while *FGFRL1*, located distal to WHSCR1/WHSCR2 and involved in bone cartilage formation during embryonic development, might be another candidate gene for craniofacial features of WHS [Catela et al., 2009; Engbers et al., 2009]. Other genes localized around these critical regions might also contribute to the various features of WHS.

Here, we present detailed clinical, microarray and FISH-based molecular-cytogenetic findings of 22 Japanese patients with WHS, representing the first large series in a non-Western country.

MATERIALS AND METHODS

Patients

Twenty-two WHS patients from eight hospitals were included in this study between January 2010 and February 2012. The diagnosis of WHS was made from clinical characteristics as well as G-banded karyotyping with or without FISH analysis using a WHSCR or 4p subtelomeric probe. Written informed consent was obtained from all parents of the patients. Clinical information was collected by the clinical geneticists of each hospital and reviewed by one of them (K.S.). Ethical approval for this study was granted by the Institutional Review Board of Shinshu University School of Medicine, Matsumoto,

Japan.

Chromosomal Microarray Analysis

Genomic DNA was isolated using standard protocols from the peripheral blood leukocytes of each patient. Chromosomal microarray analysis was performed through two whole genome oligonucleotide-based array platforms. NimbleGen CGX Array™ (Roche NimbleGen, Inc., Madison, WI) was used in the analyses of 21 patients, and includes 134,829 probes with an average resolution of 35 kb throughout the genome and 10 kb in clinically significant regions. Procedures for DNA labeling and microarray analysis were performed according to the manufacturer's instructions. The fluorescence signals on array slides were analyzed using a NimbleScan™ (Roche NimbleGen, Inc.), and presentation of array results was obtained by Genoplyphix® Software (Signature Genomics Laboratories, Spokane, WA). The Agilent Human Genome Microarray Kit 244A™ (Agilent Technologies, Santa Clara, CA) was used in the analysis of one patient, and includes 243,504 probes with a median probe space of 7.4 kb or 13.4 kb in intragenic and intergenic genomic sequences, respectively. Labeling and hybridization were performed according to the manufacturer's instructions, followed by scanning with an Agilent Microarray Scanner™ and data extraction with Feature Extraction Software™ (v9.5.3). The results were analyzed using Cytogenomics 2.0 Software™ (Agilent Technologies). Genomic coordinates of both array results were indicated according to NCBI build 36 (hg 18).

Metaphase FISH Analysis

To confirm cytogenetic rearrangements resulting in 4p deletion, FISH analysis using Bacterial Artificial Chromosome (BAC) probes was performed on metaphase chromosomes from peripheral blood leukocytes of all patients. We selected BAC probes

around deleted regions of 4p and around other terminal duplicated segments in patients who were predicted to have unbalanced rearrangements according to microarray results. Parental blood samples, where available, were also assayed with metaphase FISH to define whether chromosomal derivatives were *de novo* or inherited from parental balanced rearrangements. When a mosaic chromosomal abnormality was detected by G-banded karyotyping or suspected by chromosomal microarray results that showed a lower absolute \log_2 ratio than observed in patients with complete deletions or duplications, FISH analysis was performed on over 30 cells to detect a mosaic ratio.

RESULTS

Molecular-Cytogenetic Findings

Molecular-cytogenetic findings are summarized in Table I. G-banded chromosome analysis at the 400–550 band level, which was performed prior to this study, was abnormal in 18/22 (82%) patients: with terminal deletions of 4p in eight patients, interstitial deletions in three, additional materials of unknown origin attached to 4p in six, and mosaic chromosomes for a del(4)(p15.3p16.1) cell line (interstitial deletion) and a normal 46,XX cell line in Patient 15. G-banded chromosomes were normal in other patients who were found to have a deletion of 4p by FISH analysis using a 4p subtelomeric probe.

Chromosomal microarray analysis revealed 4p deletions to be terminal in 20 patients and interstitial in the other two patients. Deletion sizes ranged from 2.06 to 29.42 Mb, and all included WHSCR2 (Fig. 1). In patients with 4p terminal deletions, those ≤ 5.26 Mb were not detected by G-banded karyotyping. Only three patients (Patients 10, 11, and 12) shared the same breakpoints between 8.77 Mb (minimum) and 9.41 Mb (maximum) from the 4p terminus, corresponding to the loci of olfactory receptor (OR) gene clusters. Duplicated chromosomal regions accompanied by 4p deletion included 772 kb of terminal 10q in

Patient 1, 45.6 Mb of terminal 4q in Patient 3, 6.9 Mb of terminal 8p in Patient 11, and 1.27 Mb of terminal 11q in Patient 20. In Patient 20, \log_2 values for probes spanning the duplicated 11q region were approximately 0.365 (theoretical \log_2 value of non-mosaic duplication, 0.58), which suggested that the duplication was mosaic (Fig. 2A).

FISH analysis using BAC probes designed according to microarray results confirmed a derivative chromosome 4 consisting of a duplicated 4q segment on the deleted 4p in Patient 3, and unbalanced translocations between 4p and 10q in Patient 1, and between 4p and 8p in Patient 11. In Patient 20, metaphases with an unbalanced translocation, der(4)t(4;11)(p15.31;q25), were found in 22/30 cells; and those with a simple terminal deletion, del(4)(p15.31), were found in 8/30 cells. The breakpoint of 4p was considered to be identical in both cell lines (21.0 Mb from 4pter) (Fig. 2B, C). In Patient 15, metaphases with del(4)(p15.33) (11.9–12.1 Mb deletion) were found in 45/56 cells and those with del(4)(p16.3) (2.48–2.66 Mb deletion) were found in 11/56 cells (Fig. 3B, C), although only the larger deletion was demonstrated in microarray analysis (Fig. 3A). In nine patients whose parental samples were available, eight were found to have *de novo* deletions and the other (Patient 11) was found to have a maternal unbalanced translocation.

Clinical Findings and Correlation with Genotype

Clinical findings are summarized in Table II and major structural defects are listed according to deletion size in Table III. We categorized patients according to their deletion sizes into “small” with 4p terminal deletions less than 6 Mb (Patients 1–6), “intermediate” with deletions ranging from 6–15 Mb (Patients 7–17), and “large” with deletions over 15 Mb (Patients 18–22).

Neurological Features

Seizures began in 20/21 (95%) patients within the first three years of life (1 month to 2 years and 6 months old). Patient 10 was excluded because he was only 7 months old at the time of this study and might be expected to develop seizures in the future. Only Patient 13, aged 12 years with an 8.8 Mb interstitial deletion, had no seizures. The onset of seizures was late, occurring over 1 year of age, in 4/6 (67%) patients with small deletions (<6 Mb); and it was early, under 1 year of age, in 12/14 (86%) patients with larger deletions (>6 Mb). Status epilepticus occurred in 14/20 (70%) patients: 1/6 (17%) in those with small deletions and 13/14 (93%) in those with larger deletions. Seizures were intractable in six patients of ages ranging from 9 months to 6 years (median, 3 years and 7 months old), while seizures improved or disappeared in 14 patients of ages ranging from 1 year and 8 months to 18 years (median, 6 years and 2 months old). Potassium or sodium bromide was administered to four patients and the daily dose of bromide was 450mg in Patient 7, 560mg in Patient 8, 200mg in patient 14, and 400mg in Patient 18 at the time of this study. Seizures decreased in all of these. Patient 7 and Patient 8 showed a particularly obvious improvement after bromide therapy started at the ages of 1 year and 3 years, respectively. Neuroimaging demonstrated structural central nervous system defects in 9/18 (50%) patients, including periventricular leukomalacia, ventricular enlargement, hypoplasia of the corpus callosum, and cerebral or cerebellar atrophy.

A total of 19/22 (86%) patients showed severe developmental delay, while the other three patients showed a moderate delay. Independent walking was achieved in seven patients, including one with no seizures and five with no history of status epileptics.

Other Clinical Findings

Typical craniofacial features were present in all the patients except for Patient 1 with the smallest sized deletion (2.06 Mb) (Fig.4a) and Patient 13 with an interstitial deletion

(1.37-10.22 Mb) (Fig. 4h), both showing subtle features without the Greek warrior helmet appearance. The severity of short stature varied among patients regardless of the deletion sizes and the deleted regions. Eleven patients with various deletion sizes required tube feeding because of insufficient oral feeding associated with hypotonia, poorly coordinated swallowing, and/or gastroesophageal reflux. Gastrostomy was performed in two patients with the largest and the second largest deletions because of persistent insufficient oral feeding, failure to thrive regardless of tube feeding, recurrent respiratory distress, hypoglycemia, and/or carnitine deficiency.

Congenital heart defects were seen in 19/22 patients (86%). Three patients without heart defects had smaller deletions of 2.93 Mb, 5.49 Mb, and 7.51 Mb. The observed heart defects were common types, including atrial septal defects in 13 patients, pulmonary stenosis in six, and patent ductus arteriosus in five. The severity of the heart defects was not correlated with deletion sizes or deleted regions. No life-threatening complex heart defects were present in this series.

Structural urogenital anomalies were detected in a total of 47% (9/19) patients, including renal hypoplasia or dysplasia in six. The prevalence of patients with renal hypoplasia or dysplasia was 0/3 (0%) in the small deletion-type, 3/11 (27%) in the intermediate, and 3/5 (60%) in the large. Renal hypoplasia or dysplasia resulted in renal failure in five patients (83%).

Ophthalmologic abnormalities were detected in a total of 62% patients (13/21). Strabismus and nasolacrimal obstruction were found in patients with small or intermediate deletion-types, whereas structural ocular anomalies were found in patients with intermediate (2/11, 18%) and large (3/5, 60%) deletion-types. The prevalence of cleft lip/palate was 32% (7/22), that of skeletal abnormalities was 45% (9/20), and that of hearing impairment was 55% (12/22).

Other complications included hypercholesterolemia (>220 mg/dl) in a total of 36% (5/14) patients in whom serum cholesterol levels were examined, and the hypercholesterolemia was not familial in all the patients except one, whose parental information was not available. Multiple osteochondromatosis was observed in Patient 2 with a terminal 2.29 Mb deletion. The age of onset of osteochondromatosis was around 2 years. Radiological examination revealed a cartilage-capped bony growth arising from the area of the growth plate of the distal tibia and from the surface of the scapula. The patient underwent several surgical resections for progressive osteochondromatosis. Direct sequencing and multiplex ligation-dependent probe amplification analysis of *EXT1* and *EXT2*, the genes responsible for multiple osteochondromatosis (multiple exostosis type I [MIM#133700] and type II [MIM#133701], respectively) [Francannet et al., 2001], showed no pathogenic sequence variants.

DISCUSSION

In the current study, we performed microarray and FISH-based molecular-cytogenetic investigations of 22 Japanese patients with WHS, coupled with a detailed and comprehensive clinical evaluation. This resulted in identification of previously unreported complex chromosomal mosaicism and implication of several findings about the genotype-phenotype correlation including severity of seizures and structural anomalies.

Unbalanced translocations combined with a 4p deletion or other complicated rearrangement were identified in a total of four (18%) patients, which was lower than the recently reported frequency of 45% (15/33) by South et al. [2008c]. This discrepancy might be attributable to selection bias in both studies or the possible presence of a translocation with acrocentric p-arm in the current study, which could be detected through silver staining of the nucleolar organizing region (NOR), FISH using alpha satellite DNA probes, or parental FISH

studies using a WHS-specific 4p16.3 probe [South et al., 2008c].

Hitherto unreported patterns of complex mosaicism for two different structurally-abnormal cell lines were identified in two of the patients in our current series using a combination of G-banding, microarray, and metaphase FISH analysis. Only a limited number of mosaicism cases have been previously reported of two cell lines both carrying 46 chromosomes and different structurally-abnormal chromosomes, including del(8p)/inv dup del(8p) in several patients [Vermeesch et al., 2003; Pramparo et al., 2004; Hand et al., 2010]. Only one WHS patient was reported to carry two different structurally-abnormal cell lines, del(4)(p16)/der(4)(qter-q31.3::pter-qter), which might have resulted from a meiotic crossing over event causing the der(4) cell line to be associated with a pericentric inversion and subsequent mitotic breakage [Syrrou et al., 2001]. A previous report showed expansion of a 4p terminal deletion between a mother and a son [Faravelli et al., 2007] and a subsequent report described this on 18q [South et al., 2008b]. Patient 15 might be another example of apparent instability of a terminal deletion, representing the expansion of a deletion within an individual rather than between generations.

Mosaicism of two different structurally-abnormal cell lines, del(4)(p15.31)/der(4)t(4;11)(p15.31;q25), was indicated in Patient 20 by microarray through the lower \log_2 ratio of the duplicated 11q region. This supports the utility of microarray in that it can detect not only small copy number variation at a significantly higher resolution, but also detect mosaicism by incomplete \log_2 ratio compared with complete deletion or duplication [Ballif et al., 2006]. By contrast, mosaicism could not be detected in Patient 15 by microarray, perhaps because of the different levels of mosaicism between the two patients: 22:8 in Patient 20 and 45:11 in Patient 15.

Our study included four patients with other duplicated chromosomal regions detected by microarray. The duplicated segment of 10q26.3–qter (772 kb) in Patient 1, 11q25–qter

(1.27 Mb, mosaicism) in Patient 20, and 8p23.1–pter (6.9 Mb) in Patient 11 have not been reported to associate with extensive disease pathology in a trisomic state [Engelen et al.2000; Harada et al., 2002; Iwanowski et al.2011]. The 45.6 Mb duplication at the 4q31.22–qter region in Patient 3 is considered to be mainly associated with psychomotor delay and often with cardiac and renal anomalies [Otsuka et al., 2005; Wang et al., 2009]. Indeed, Patient 3 showed severe developmental delay in spite of a small 4p deletion (2.93 Mb), but no apparent cardiac or renal anomaly.

The severity of seizures is evaluated from the time of onset and the presence of status epilepticus. Six patients with small deletions (<6 Mb) from 4pter tended to have a later onset of seizures and status epilepticus was less common than those patients with intermediate (6–15 Mb) or large deletions (>15 Mb). Developmental delay was severe in most patients, with the exception of three with a moderate delay: two of these had small terminal deletions (2.06 Mb and 5.26 Mb) and one had an intermediate interstitial deletion (8.85 Mb). Seizure severity is, therefore, suggested to correlate with the 4p deletion size, which might result in correlation between severity of developmental delay and the 4p deletion size.

Candidate region(s) for seizures in patients with WHS and possible responsible genes are shown in Fig. 5. Although *LETMI* is presently considered to be the major responsible gene for seizures [Endele et al., 1999; Rauch et al., 2001; South et al., 2007], the more distal region of the chromosome has also been suggested as a candidate region for seizure penetrance [South et al., 2008a; Misceo et al., 2012]. Indeed, Patient 13 in our series did not have seizures and had an interstitial deletion (1.37–10.22 Mb from 4pter) encompassing *LETMI* but preserving the distal regions, which is similar to “Case 6” reported by Maas et al. [2008] with an interstitial deletion (1.3–2.5Mb) including *LETMI* and no seizures. Four patients with seizures were reported to have small distal 4p deletions

not including *LETMI* [Faravelli et al., 2007; Maas et al., 2008; Zollino et al., 2008; Misceo et al., 2012]. Considering a patient with ring chromosome 4 and a 4p terminal deletion of 760 kb not experiencing seizures [Concolino et al., 2007], the susceptible gene(s) for seizures in WHS might be localized in the region between 760 kb and 1.3 Mb from the 4pter. In our series, Patient 17 with an interstitial deletion encompassing both *LETMI* and most of the new candidate region had severe seizures.

CTBP1 and *CPLX1* are localized in this new susceptible region for seizures. *CTBP1* encodes a transcriptional corepressor that acts at the promoters of many genes [Chinnadurai, 2007]. In an epileptogenic rat model, a ketogenic diet as well as 2-deoxy-D-glucose, a glycolysis-inhibiting drug, reduce epilepsy by stimulating Ctbp activity. Ctbp co-operates with transcriptional factor NRSF to repress expression of BDNF, a strongly suspected epileptogenic signaling molecule [Garriga-Canut et al., 2006]. The hemizyosity of *CTBP1* in WHS patients is therefore considered a potential contributor to the progression of epilepsy [Simon and Bergemann, 2008]. *CPLX1* encodes a type of complexin that binds to syntaxin within the SNARE complex and regulates the fusion of synaptic vesicles [McMahon et al., 1995]. Homozygous *Cplx1* deletion mutant mice develop strong ataxia and sporadic seizures [Reim et al., 2001; Glynn et al., 2005]. These findings suggest that *CTBP1* and *CPLX1* as well as *LETMI* could be susceptibility genes for seizures in WHS.

Bromide therapy was previously reported to be an effective antiepileptic drug in four patients with WHS, in whom it was shown to reduce status epilepticus [Kagitani-Shimono et al., 2005]. In the current study, four patients were administered bromide therapy, which was effective in all. In particular, Patients 7 and 8 showed a marked reduction in seizure frequency after the initiation of bromide therapy. Further information including the types or severity of seizures, electroencephalography (EEG) patterns, efficacy of treatment, and microarray-based deletion mapping in a larger patient series will be necessary to establish a

detailed seizure phenotype-genotype correlation.

Hypercholesterolemia, which has not been reported in previous studies, was observed in five patients in the present study, suggesting it to be a noteworthy complication of WHS. *LRPAP1*, localized 3.5 Mb from 4pter, was deleted in four of the patients. *LRPAP1* encodes LDL receptor-related protein-associated protein 1 that plays an important role in lipoprotein metabolism [Willnow et al., 1995], and might therefore be related to hypercholesterolemia. Multifactorial inheritance, including nutritional problems, could also be related to the occurrence of hypercholesterolemia.

In conclusion, this genotype-phenotype correlation study using microarray and FISH-based molecular-cytogenetic investigations uncovered chromosomal rearrangements in all patients including previously unreported complex chromosomal mosaicism. It also demonstrated the correlation of deletion size from 4pter with seizure severity and with occurrence of renal hypoplasia/dysplasia and structural ocular anomalies, and described additional clinical features including hypercholesterolemia. Moreover, a new susceptible region distal to the previously-supposed candidate gene *LETMI* was suggested for the occurrence of seizures, and the usefulness of bromide therapy was stressed for seizure management. To prevent intractable seizures and status epileptics, patients with 4p deletion involving the new susceptible region as well as *LETMI* are recommended to have careful EEG follow-up and intensive pharmacological treatment based on the seizure occurrence and EEG findings, including application of bromide therapy. These findings are relevant to the improvement of WHS healthcare guidelines, as well as to the elucidation of gene(s) function in the deleted region.

ACKNOWLEDGMENTS

We thank the patients and their families for participating in this study. This work was

supported by Research on Intractable Diseases from Japanese Ministry of Health, Labour and Welfare (K.T., Y.F.).

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES

- Ballif BC, Rorem EA, Sundin K, Lincicum M, Gaskin S, Coppinger J, Kashork CD, Shaffer LG, Bejjani BA. 2006. Detection of low-level mosaicism by array CGH in routine diagnostic specimens. *Am J Med Genet A* 140:2757–2767.
- Battaglia A, Carey JC, Cederholm P, Viskochil DH, Brothman AR, Galasso C. 1999. Natural history of Wolf-Hirschhorn syndrome: experience with 15 cases. *Pediatrics* 103:830–836.
- Battaglia A, Filippi T, Carey JC. 2008. Update on the clinical features and natural history of Wolf-Hirschhorn (4p-) syndrome: experience with 87 patients and recommendations for routine health supervision. *Am J Med Genet Part C* 148C:246–251.
- 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.
- Catela C, Bilbao-Cortes D, Slonimsky E, Kratsios P, Rosenthal N, Te Welscher P. 2009. Multiple congenital malformations of Wolf-Hirschhorn syndrome are recapitulated in *Fgfr1* null mice. *Dis Model Mech* 2:283–294.
- Chinnadurai G. 2007. Transcriptional regulation by C-terminal binding proteins. *Int J Biochem Cell Biol* 39:1593–1607.
- Concolino D, Rossi E, Strisciuglio P, Iembo MA, Giorda R, Ciccone R, Tenconi R, Zuffardi O. 2007. Deletion of a 760 kb region at 4p16 determines the prenatal and postnatal growth retardation characteristic of Wolf-Hirschhorn syndrome. *J Med Genet* 44:647–650.
- Endele S, Fuhry M, Pak SJ, Zabel BU, Winterpacht A. 1999. LETM1, a novel gene encoding a putative EF-hand Ca(2+)-binding protein, flanks the Wolf-Hirschhorn syndrome (WHS) critical region and is deleted in most WHS patients. *Genomics*

60:218–225.

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.

Engelen JJ, Moog U, Evers JL, Dassen H, Albrechts JC, Hamers AJ. 2000. Duplication of chromosome region 8p23.1-->p23.3: a benign variant? *Am J Med Genet* 91:18–21.

Faravelli F, Murdolo M, Marangi G, Bricarelli FD, Di Rocco M, Zollino M. 2007. Mother to son amplification of a small subtelomeric deletion: a new mechanism of familial recurrence in microdeletion syndromes. *Am J Med Genet Part A* 143A:1169–1173.

Francannet C, Cohen-Tanugi A, Le Merrer M, Munnich A, Bonaventure J, Legeai-Mallet L. 2001. Genotype-phenotype correlation in hereditary multiple exostoses. *J Med Genet* 38:430–434.

Garriga-Canut M, Schoenike B, Qazi R, Bergendahl K, Daley TJ, Pfender RM, Morrison JF, Ockuly J, Stafstrom C, Sutula T, Roopra A. 2006. 2-Deoxy-D-glucose reduces epilepsy progression by NRSF-CtBP-dependent metabolic regulation of chromatin structure. *Nat Neurosci* 9:1382–1387.

Glynn D, Drew CJ, Reim K, Brose N, Morton AJ. 2005. Profound ataxia in complexin I knockout mice masks a complex phenotype that includes exploratory and habituation deficits. *Hum Mol Genet* 14:2369–2385.

Hand M, Gray C, Glew G, Tsuchiya KD. 2010. Mild phenotype in a patient with mosaic del(8p)/inv dup del(8p). *Am J Med Genet Part A* 152A:2827–2831.

Harada N, Takano J, Kondoh T, Ohashi H, Hasegawa T, Sugawara H, Ida T, Yoshiura K, Ohta T, Kishino T, Kajii T, Niikawa N, Matsumoto N. 2002. Duplication of 8p23.2: a benign cytogenetic variant? *Am J Med Genet* 111:285–288.

- Hirschhorn K, Cooper HL, Firschein IL. 1965. Deletion of short arms of chromosome 4-5 in a child with defects of midline fusion. *Humangenetik* 1:479–482.
- Iwanowski PS, Panasiuk B, Van Buggenhout G, Murdolo M, Mysliwiec M, Maas NM, Lattante S, Korniszewski L, Posmyk R, Pilch J, Zajaczek S, Fryns JP, Zollino M, Midro AT. 2011. Wolf-Hirschhorn syndrome due to pure and translocation forms of monosomy 4p16.1 --> pter. *Am J Med Genet Part A* 155A:1833–1847.
- 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.
- Jiang D, Zhao L, Clapham DE. 2009. Genome-wide RNAi screen identifies *Letm1* as a mitochondrial $\text{Ca}^{2+}/\text{H}^{+}$ antiporter. *Science* 326:144–147.
- Kagitani-Shimono K, Imai K, Otani K, Kamio N, Okinaga T, Toribe Y, Suzuki Y, Ozono K. 2005. Epilepsy in Wolf-Hirschhorn syndrome (4p-). *Epilepsia* 46:150–155.
- Maas NM, Van Vooren S, Hannes F, Van Buggenhout G, Mysliwiec M, Moreau Y, Fagan K, Midro A, Engiz O, Balci S, Parker MJ, Sznajder Y, Devriendt K, Fryns JP, Vermeesch JR. 2007. The t(4;8) is mediated by homologous recombination between olfactory receptor gene clusters, but other 4p16 translocations occur at random. *Genet Couns* 18:357–365.
- 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.
- McMahon HT, Missler M, Li C, Sudhof TC. 1995. Complexins: cytosolic proteins that

- regulate SNAP receptor function. *Cell* 83:111–119.
- Misceo D, Baroy T, Helle JR, Braaten O, Fannemel M, Frengen E. 2012. 1.5Mb deletion of chromosome 4p16.3 associated with postnatal growth delay, psychomotor impairment, epilepsy, impulsive behavior and asynchronous skeletal development. *Gene* 507:85–91.
- Nimura K, Ura K, Shiratori H, Ikawa M, Okabe M, Schwartz RJ, Kaneda Y. 2009. A histone H3 lysine 36 trimethyltransferase links Nkx2-5 to Wolf-Hirschhorn syndrome. *Nature* 460:287–291.
- Otsuka T, Fujinaka H, Imamura M, Tanaka Y, Hayakawa H, Tomizawa S. 2005. Duplication of chromosome 4q: renal pathology of two siblings. *Am J Med Genet A* 134:330–333.
- Pramparo T, Giglio S, Gregato G, de Gregori M, Patricelli MG, Ciccone R, Scappaticci S, Mannino G, Lombardi C, Pirola B, Giorda R, Rocchi M, Zuffardi O. 2004. Inverted duplications: how many of them are mosaic? *Eur J Hum Genet* 12:713–717.
- Rauch A, Schellmoser S, Kraus C, Dorr HG, Trautmann U, Altherr MR, Pfeiffer RA, Reis A. 2001. First known microdeletion within the Wolf-Hirschhorn syndrome critical region refines genotype-phenotype correlation. *Am J Med Genet* 99:338–342.
- Reim K, Mansour M, Varoquaux F, McMahon HT, Sudhof TC, Brose N, Rosenmund C. 2001. Complexins regulate a late step in Ca²⁺-dependent neurotransmitter release. *Cell* 104:71–81.
- Rodriguez L, Zollino M, Climent S, Mansilla E, Lopez-Grondona F, Martinez-Fernandez ML, Murdolo M, Martinez-Frias 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.
- Simon R, Bergemann AD. 2008. Mouse models of Wolf-Hirschhorn syndrome. *Am J Med Genet Part C* 148C:275–280.

- 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. 2008a. Pathogenic significance of deletions distal to the currently described Wolf-Hirschhorn syndrome critical regions on 4p16.3. *Am J Med Genet Part C* 148C:270–274.
- South ST, Rope AF, Lamb AN, Aston E, Glaus N, Whitby H, Maxwell T, Zhu XL, Brothman AR. 2008b. Expansion in size of a terminal deletion: a paradigm shift for parental follow-up studies. *J Med Genet* 45:391–395.
- South ST, Whitby H, Battaglia A, Carey JC, Brothman AR. 2008c. Comprehensive analysis of Wolf-Hirschhorn syndrome using array CGH indicates a high prevalence of translocations. *Eur J Hum Genet* 16:45–52.
- Syrrou M, Borghgraef M, Fryns JP. 2001. Unusual chromosomal mosaicism in Wolf-Hirschhorn syndrome: del(4)(p16)/der(4)(qter-q31.3::pter-qter). *Am J Med Genet* 104:199–203.
- Vermeesch JR, Thoelen R, Salden I, Raes M, Matthijs G, Fryns JP. 2003. Mosaicism del(8p)/inv dup(8p) in a dysmorphic female infant: a mosaic formed by a meiotic error at the 8p OR gene and an independent terminal deletion event. *J Med Genet* 40:e93.
- Wang JC, Fisker T, Dang L, Teshima I, Nowaczyk MJ. 2009. 4.3-Mb triplication of 4q32.1-q32.2: report of a family through two generations. *Am J Med Genet Part A* 149A:2274–2279.
- Willnow TE, Armstrong SA, Hammer RE, Herz J. 1995. Functional expression of low density lipoprotein receptor-related protein is controlled by receptor-associated protein in vivo. *Proc Natl Acad Sci USA* 92:4537–4541.
- Wolf U, Reinwein H, Porsh R, Schroter R, Baitsch H. 1965. Defizienz am den kurze

Armen eines chromosomes nr. 4. Humnangenetik 1:397–413.

Wright TJ, Ricke DO, Denison K, Abmayr S, Cotter PD, Hirschhorn K, Keinanen 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, Di Stefano C, Zampino G, Mastroiacovo P, Wright TJ, Sorge G, Selicorni A, Tenconi R, Zappala A, Battaglia A, Di Rocco M, Palka G, Pallotta R, Altherr MR, Neri G. 2000. Genotype-phenotype correlations and clinical diagnostic criteria in Wolf-Hirschhorn syndrome. *Am J Med Genet* 94:254–261.

Zollino M, Lecce R, Fischetto R, Murdolo M, Faravelli F, Selicorni A, Butte 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* 148C:257–269.

Figure Legends

FIG. 1. Overview of 4p deletion sizes in each patient using chromosomal microarray analysis. Gray bars indicate deleted segments, black boxes indicate WHSCR2, and # denotes presence of other unbalanced regions. *Patient 15 has a mosaicism with another minor cell line of a smaller-sized terminal deletion determined by metaphase FISH.

FIG. 2. Molecular-cytogenetic investigations of Patient 20.

A: Upper panel shows microarray results. Whereas a 21.0 Mb copy number loss of 4pter with an average \log_2 value of -0.775 does not show a mosaicism, a 1.27 Mb copy number gain of 11qter with an average \log_2 value of 0.365 suggests a mosaicism. Lower panel shows BAC probes used in metaphase FISH. 4p probes deleted in both cell lines are shown in red, 11q probes duplicated in only the der(4)t(4;11) cell line are shown in green, and adjacent probes not deleted or duplicated in either cell line are shown in yellow at 4p or white at 11q. B: Metaphase FISH analysis showing the der(4)t(4;11) cell line. C: Metaphase FISH analysis showing the del(4) cell line. RP11-264K20 probe is shown in red, that for RP11-92M20 in yellow, and that for RP11-296M15 in green.

FIG. 3. Molecular-cytogenetic investigations of Patient 15.

A: Upper panel shows microarray results. A 15 Mb copy number loss with an average \log_2 value of -0.764 does not suggest a mosaicism. Lower panel shows BAC probes used in metaphase FISH. Those deleted in both cell lines are shown in yellow, those deleted only in the cell line of the larger deletion are shown in red, and those not deleted in either cell line are shown in green. B: Metaphase FISH analysis showing the del(4)(p16.3) (2.48–2.66 Mb deletion) cell line. C: Metaphase FISH analysis showing the del(4)(p15.33) (11.9–12.1 Mb deletion) cell line. RP11-116N15 probe is shown in yellow, that for RP11-118L8 in red,

and that for RP11-652B7 in green.

FIG. 4. Clinical photographs. Patient 1 at age 8 years and 10 months (a), Patient 2 at age 4 years (b), Patient 6 at age 18 years (c), Patient 7 at age 2 years and 2 months (d), Patient 8 at age 3 months (e), Patient 11 at age 8 years and 8 months (f), Patient 12 at age 1 year and 3 months (g), Patient 13 at age 6 years and 4 months (h), and Patient 16 at age 2 years and 3 months (i). Typical craniofacial features are present in all the patients except for Patient 1 (a) and Patient 13 (h), showing subtle features without the Greek warrior helmet appearance.

FIG. 5. Schema showing candidate region(s) for the occurrence of seizures [Genome coordinates hg18]. A new candidate region is suggested between 0.76–1.3 Mb from 4pter, encompassing *CTBPI* and *CPLX1*, and distal to the previously-susposed candidate gene *LETMI*. White bars indicate deleted segments in patients without seizures. Light gray bars indicate deleted segments in those with seizures. Bars with horizontal stripes indicate deletion breakpoints with variation according to probe-gaps. # represents patients in whom the breakpoint was determined by oligonucleotide microarray, while breakpoints in the other patients were determined by BAC array or FISH using BAC probes.

TABLE I. Summary of Molecular-Cytogenetic Analysis of 22 WHS Patients

Patient No.	G-banded chromosomes	Array-CGH analysis					Final 4p rearrangement studied with a-CGH and FISH	Inheritance
		Location and minimal deletion size of 4p	Distal breakpoint from 4pter#	Proximal breakpoint from 4pter	Other unbalanced region and minimal size			
1	46,XX	4p16.3	2.06 Mb	1-(63,075)	2,061,942-2,071,068	dup(10)(q26.3), 772 kb	unbalanced translocation der(4)t(4;10)(p16.3,q26.3)	
2	46,XX	4p16.3	2.29 Mb	1-(41,413)	2,294,435-2,313,104		isolated terminal	
3	add(4)(p16)	4p16.3	2.93 Mb	1-(63,075)	2,934,119-2,941,688	dup(4)(q31.22q35.2), 45.6 Mb	der(4)(4qter→q31.22;p16.3→qter)	
4	46,XX	4p16.3p16.2	3.45 Mb	1-(63,075)	3,453,423-3,475,088		isolated terminal	
5	46,XX	4p16.3p16.1	5.26 Mb	1-(63,075)	5,259,705-5,286,063		isolated terminal	
6	del(4)(p16.1)	4p16.3p16.1	5.49 Mb	1-(33,860)	5,488,869-5,517,610		isolated terminal	de novo
7	del(4)(p16.1)	4p16.3p16.1	6.92 Mb	1-(33,860)	6,920,095-6,944,615		isolated terminal	
8	del(4)(p16.1)	4p16.3p16.1	7.51 Mb	1-(63,075)	7,509,074-7,525,657		isolated terminal	
9	del(4)(p15.3p16)	4p16.3p16.1	8.09 Mb	1-(63,075)	8,089,462-8,126,238		isolated terminal	
10	add(4)(p15.3)	4p16.3p16.1	8.77 Mb	1-(63,075)	8,772,114-9,414,321		isolated terminal	
11	add(4)(p15.2)	4p16.3p16.1	8.77 Mb	1-(33,860)	8,772,114-9,414,321	dup(8)(p23.3p23.1), 6.9 Mb	unbalanced translocation der(4)t(4;8)(p16.1;p23.1)	maternal
12	del(4)(p16.1)	4p16.3p16.1	8.77 Mb	1-(33,860)	8,772,114-9,414,321		isolated terminal	de novo
13	add(4)(p15.3)	4p16.3p16.1	8.85 Mb	1,329,023-1,370,178	10,219,850-10,254,956		isolated interstitial	
14	add(4)(p15.2)	4p16.3p15.33	11.11 Mb	1-(63,075)	11,105,238-11,129,635		isolated terminal	de novo
15	46,XY,del(4)(p15.3p16.1)[26]/46,XY[4]	4p16.3p15.33	12.01 Mb	1-(33,860)	12,006,591-12,044,138		mos.del(4)(p15.33)/del(4)(p16.3)	
16	del(4)(p15.3)	4p16.3p15.33	12.33 Mb	1-(33,860)	12,331,994-1,2371,059		isolated terminal	de novo
17	del(4)(p15.32p16.3)	4p16.3p15.33	13.63 Mb	813,367-841,095	14,467,735-14,498,501		isolated interstitial	de novo
18	add(4)(p15.2)	4p16.3p15.32	15.70 Mb	1-(63,075)	15,700,625-15,737,006		isolated terminal	
19	del(4)(p15.3)	4p16.3p15.31	18.62 Mb	1-(63,075)	18,616,970-18,655,860		isolated terminal	de novo
20	del(4)(p15.2p16)	4p16.3p15.31	21.00 Mb	1-(63,075)	20,992,651-21,031,043	dup(11)(q25), 1.27 Mb	mos.der(4)t(4;11)(p15.31;q25) /del(4)(p15.31)	de novo
21	del(4)(p15.1)	4p16.3p15.1	28.35 Mb	1-(33,860)	28,348,051-28,384,613		isolated terminal	de novo
22	del(4)(p15.1)	4p16.3p15.1	29.42 Mb	1-(63,075)	29,416,450-29,451,155		isolated terminal	

Genomic locations of array results are according to NCBI build 36 (hg 18).

Distal breakpoints of 41,413, 33,860 and 63,075 actually show terminal 4p deletions because the probes designed for the region are nonspecific to 4pter.

TABLE II. Summary of Clinical Features in 22 WHS Patients

Patient No.	Age/Sex	Minimal deletion sizes in 4p(Mb)	Accompanied duplicated regions	Seizure Onset	Status Epilepticus	Treatment at the time of this study /Course of seizures	CNS complications	Developmental delay
1	6y/F	2.06	dup(10q),772 kb	+9m	–	CZP/disappeared	–	Moderate (DQ41)/walk at 2y3m
2	13y/F	2.29		+2y6m	–	VPA/disappeared for 5 years	N.I.	Severe (IQ23)/walk at 7y
3	1y8m/F	2.93	dup(4q),45.6 Mb	+1y3m	–	–/disappeared after only one attack	–	Severe/no sitting
4	4y3m/F	3.45		+7m	+	VPA,CLB/occurred frequently	Periventricular leukomalacia	Severe /no head control
5	5y10m/F	5.26		+1y1m	–	CZP/well-controlled	N.I.	Moderate/walk at 4y
6	16y/F	5.49		+2y1m	–	DZP/disappeared since 8y	N.I.	Severe/walk at 7y
7	18y/F	6.92		+6m	+	VPA,Br,Vit B6 (West syndrome) /improved after Br at 1y	–	Severe (IQ \leq 10)/walk at 7y
8	6y6m/M	7.51		+7m	+	TPM,PB,CLB,Br /improved after Br at 3y	N.I.	Severe/roll over at 1y5m
9	5y3m/F	8.09		+10m	+	PB,VPA/occurred frequently	–	Severe/head control at 12m
10	7m/M	8.77		–	–	–/–	–	Severe
11	8y/F	8.77	dup(8p),6.9 Mb	+6m	+	VPA,PB/improved after 2y	–	Severe (DQ \leq 10)/sit at 4y
12	16y/F	8.77		+7m	+	VPA,PHT /improved after 2y	Ventricular enlargement	Severe/ head control at 2y
13	12y/F	8.85 (1.37–10.22)		–	–	–/–	–	Moderate/walk at 2y3m
14	5y/F	11.11		+9m	+	VPA,Br/well-controlled	–	Severe/walk
15	9m/M	12.02		+8m	+	VPA/continued	Ventricular enlargement	Severe
16	1y11m/F	12.33		+9m	+	VPA,LGT,CZP/occurred frequently	HCC/Cerebellar atrophy	Severe (DQ30)/head control at 9m
17	18y/F	13.63 (0.84–14.5)		+1y	+	–/disappeared for several years	HCC	Severe (IQ \leq 10)/walk at 5y
18	3y/F	15.70		+1y2m	+	VPA,CLB,Br/improved gradually	–	Severe/head control
19	2y11m/F	18.60		+2m	+	PB,CZP,TPM/occurred frequently	–	Severe/no head control
20	4y/F	21.00	dup(11q),1.27 Mb	+1m	–	VPA,ZNS/disappeared for two years	Cerebral atrophy	Severe/no head control
21	2y10m/F	28.35		+11m	+	TPM,CLB/disappeared since 2y	Cerebral atrophy	Severe
22	6y/M	29.42		+2m	+	VPA,ESM,PB/occurred frequently	HCC Grey matter heterotopia White matter volume loss	Severe (DQ \leq 10) /no head control

Br, potassium/sodium bromide; CLB, clobazam; CNS, central nervous system; CZP, clonazepam; DQ, developmental quotient; DZP, diazepam; ESM, ethosuximide; F, Female; HCC, hypoplasia of the corpus callosum; IQ, intelligence quotient; LGT, lamotrigine, M, Male; m, month; N.I., not investigated; PB, phenobarbital; PHT, phenytoin; TPM, topiramate; VPA, valproate; y, years; ZNS, zonisamide.

TABLE II. Summary of Clinical Features in 22 WHS Patients (Continued)

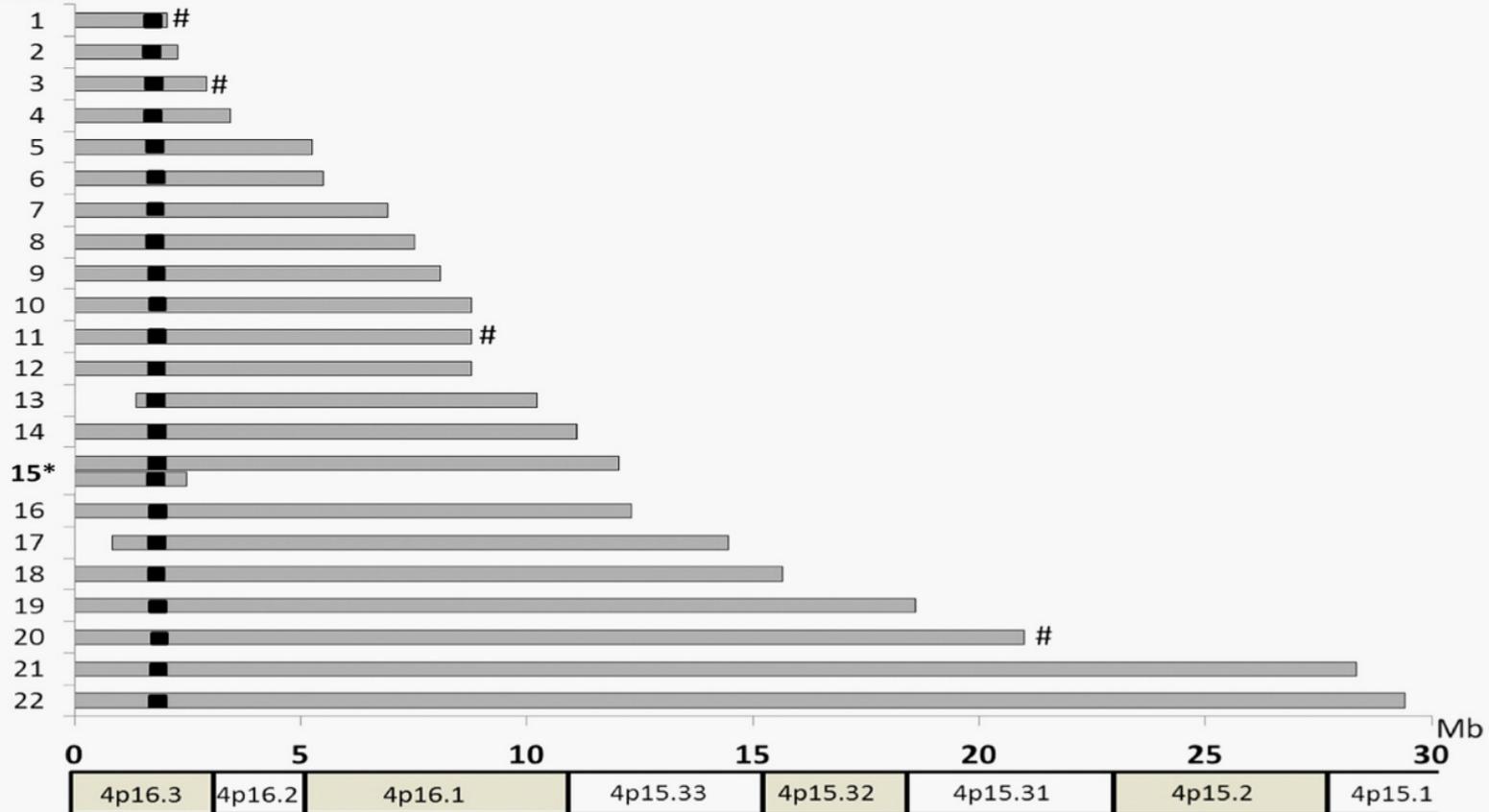
Patient No.	Height/Weight (SD)#	Feeding	CL/CP	Heart	Urogenital	Skeletal	Ophthalmologic	Hearing impairment	Other complication(s)
1	-2.4/-2.0	Oral	-	ASD	-	-	Strabismus	-	
2	-5.4/-3.5	Oral	-	ASD	N.I.	Scoliosis (mild)	-	-	Multiple osteochondromatosis
3	-5.3/-2.3	Tube	-	-	-	-	-	Severe	Hypercholesterolemia
4	-4.3/-3.0	Tube	-	ASD /PDA/VSD	N.I.	Limited hip flexion	Strabismus	Moderate	
5	-3.8/-2.9	Oral	-	ASD	N.I.	-	Strabismus	-	Hypercholesterolemia
6	-4.9/-3.0	Oral	-	-	-	N.I.	Strabismus (ET)	-	
7	-5.1/-2.5	Oral	-	PS	Renal hypoplasia/RF	Pes planus	-	Moderate	Hypercholesterolemia /Hyperuricemia
8	-6.4/-3.7	Tube	-	-	-	-	NDO	Moderate	Fanconi syndrome due to VPA
9	-5.8/-4.1	Oral	-	ASD/PS	-	-	Strabismus	Moderate	
10	-4.5/-3.9	Tube	+ (CP)	PDA	-	-	Right cataract	-	
11	-4.1/-3.3	Tube	+ (SMCP)	AR	-	N.I.	Strabismus/NDO	-	
12	-11.7/-5.3	Tube	+ (CL/CP)	PS	Renal hypoplasia/RF	Scoliosis (mild)	Strabismus (XT)	Severe	
13	-3.0/-2.3	Tube	-	VSD	-	-	-	-	
14	-5.2/-4.0	Oral	+	ASD	-	-	-	Moderate	
15	-2.0/-2.3	Oral	-	ASD/PS	Cryptorchidism	-	N.I.	Moderate	
16	-3.4/-2.7	Tube	+ (SMCP)	ASD/PDA	Hydronephrosis	Talipes varus	Strabismus (XT)/NDO	-	
17	-5.3/-3.8	Oral	-	ASD	Renal hypoplasia /RF	Acetabular dysplasia	Cataract	-	Hypercholesterolemia
18	-5.6/-4.0	Tube	-	ASD/PS	Renal hypoplasia /RF	Sagittal craniosynostosis	Coloboma	-	Hypercholesterolemia
19	-4.4/-2.4	Tube	-	ASD/PS	-	Scoliosis	Optic nerve atrophy	Moderate	
20	-3.3/-2.6	Tube	-	PDA/VSD	Renal hypoplasia VUR	-	-	Moderate	
21	-2.3/-3.1	GS	+(CP)	ASD/PDA	UJS / RF	Talipes varus Cervical spine abnormalities	-	Severe	
22	-1.3/-1.4	GS	+	ASD	Renal dysplasia/ RF Cryptorchidism Hypospadias	-	Cataract/Coloboma	Severe	

#, height and weight were evaluated at the time of this study. ASD, atrial septal defect; ET, esotropia; CL, cleft lip; CP, cleft palate; GS, gastrostomy; NDO, nasolacrimal duct obstruction; PDA, patent ductus arteriosus; PS, pulmonary stenosis; RF, renal failure; SMCP, submucous cleft palate; UJS, ureteropelvic junction stenosis; VSD, ventricular septal defect; VUR, vesicoureteric reflux; XT, exotropia.

TABLE III. Frequency of major structural defects according to deletion sizes

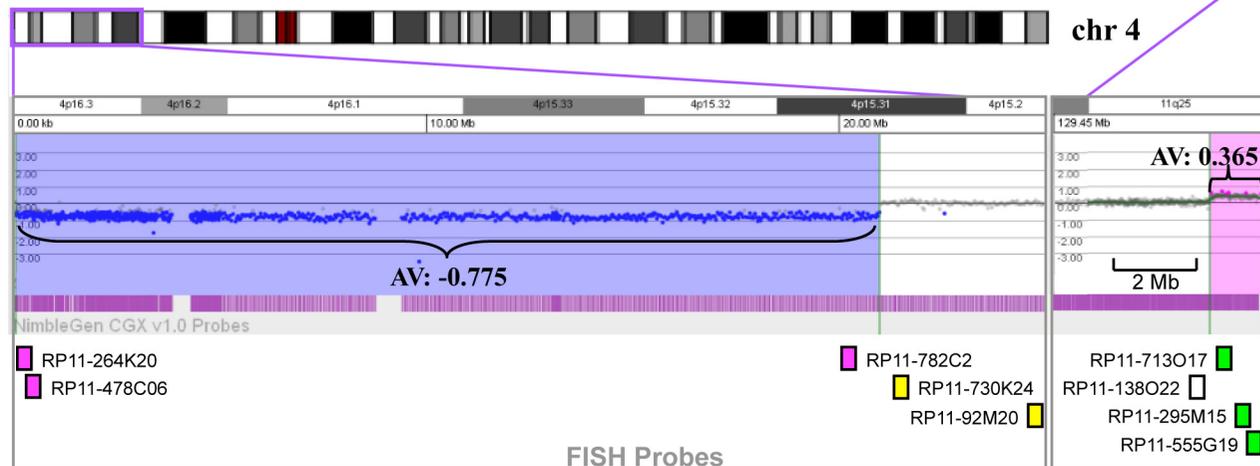
	Small (<6 Mb)	Intermediate (6–15 Mb)	Large (>15 Mb)
Congenital heart defects	4/6 (67%)	10/11 (91%)	5/5 (100%)
Renal abnormalities [Renal failure]	0/3(0%) [0/3]	4/11 (36%) [3/11]	4/5 (80%) [3/5]
Ocular defects	0/6 (0%)	2/11 (18%)	3/5 (60%)
Cleft lip/palate	0/6 (0%)	5/11 (45%)	2/5 (40%)
Skeletal anomalies	2/6 (33%)	4/11 (36%)	3/5 (60%)

Patient

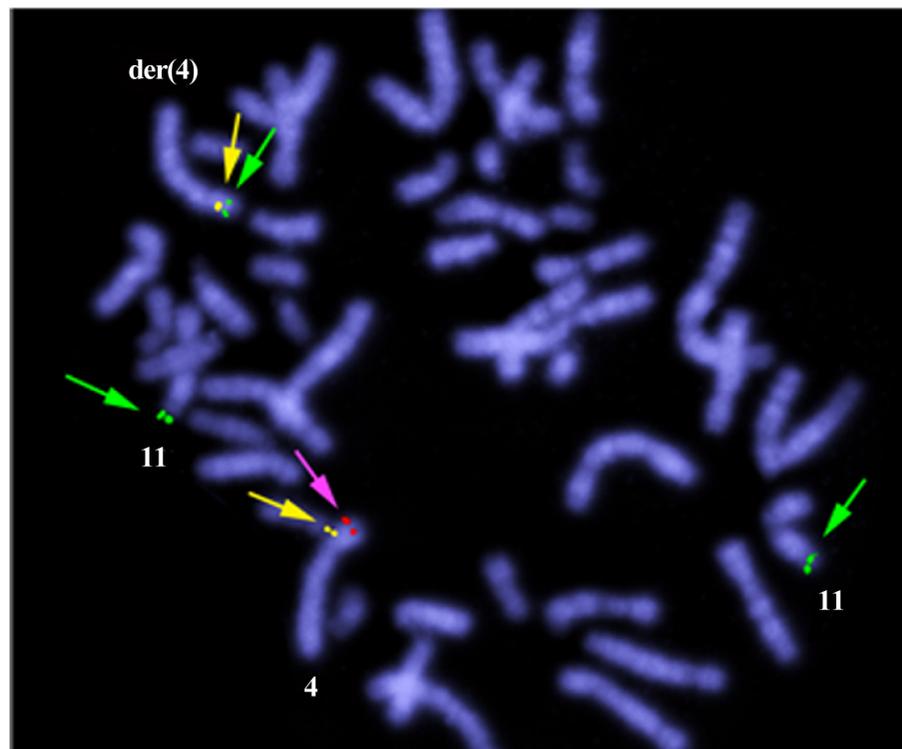


chr 11

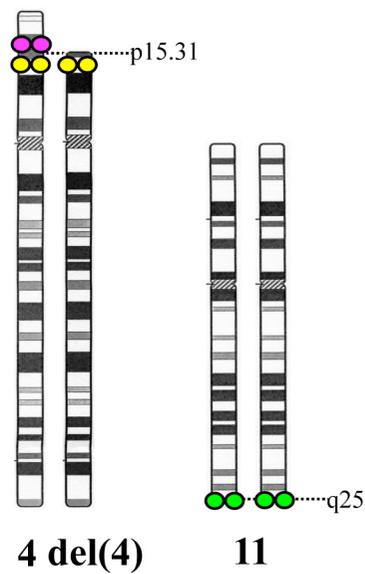
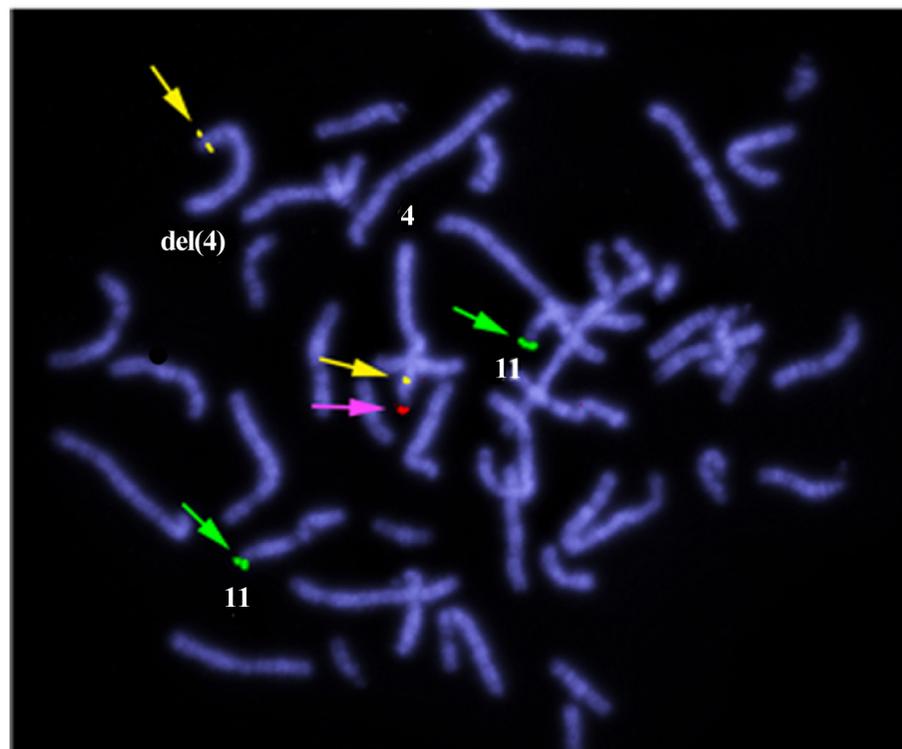
chr 4



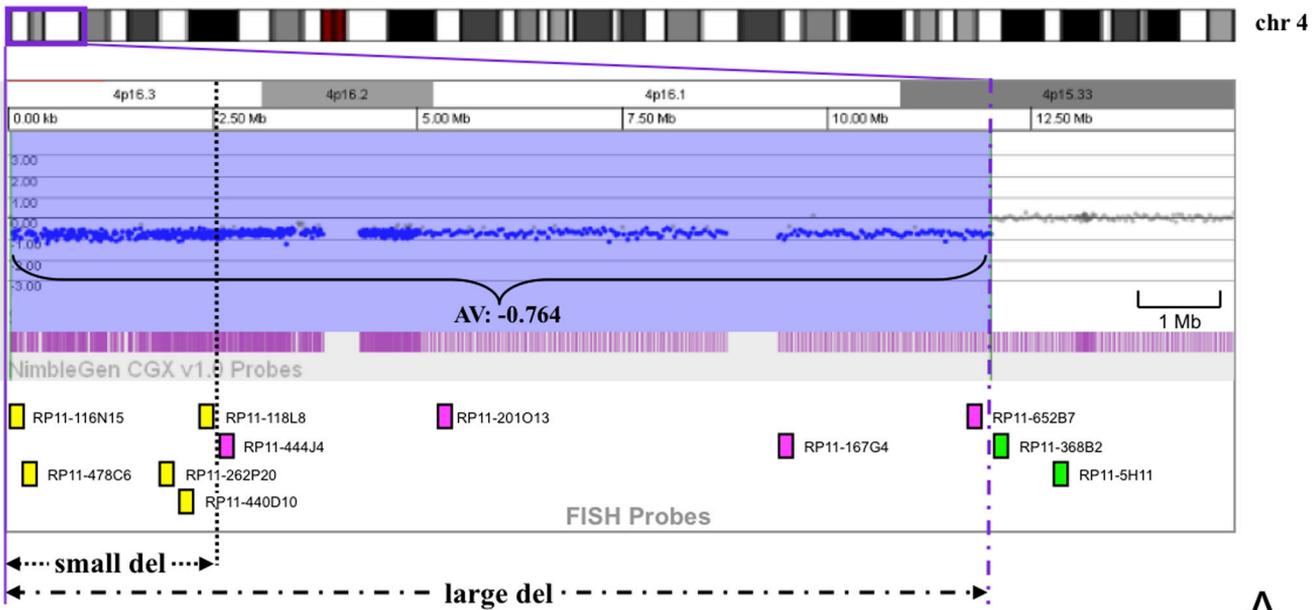
A



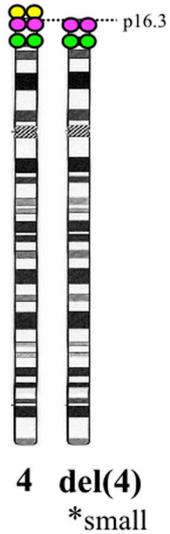
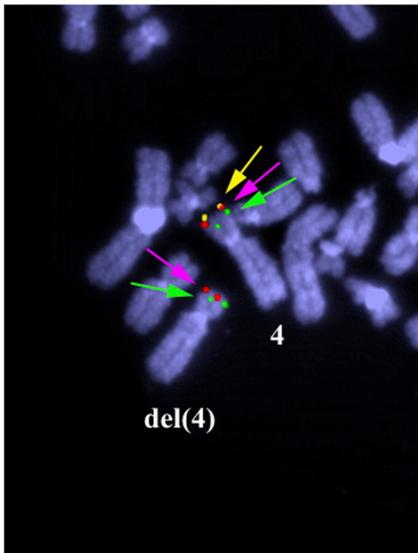
B



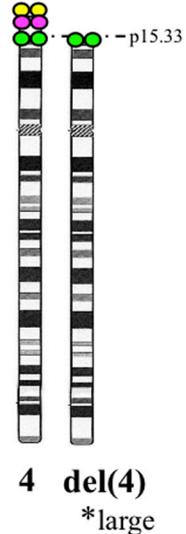
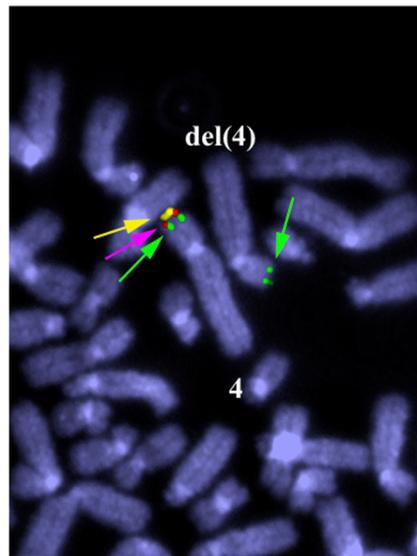
C



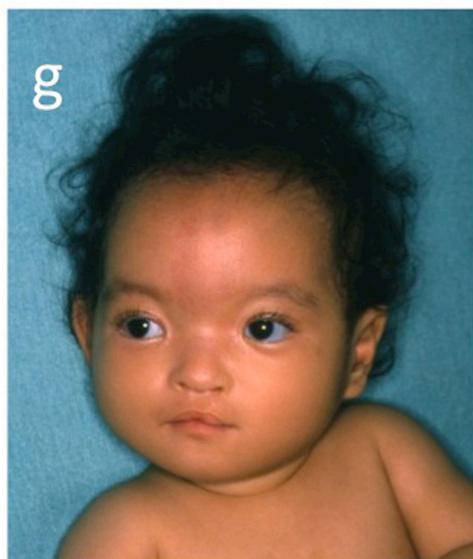
A



B

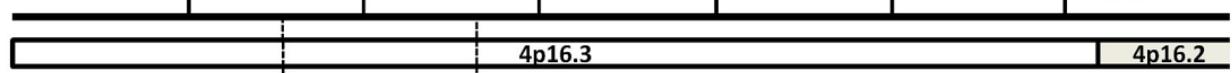


C



0 0.5 1.0 1.5 2.0 2.5 3.0 Mb

Genome position
Chromosomal band



Primary Genes

