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.
© 2013 Wiley Periodicals, Inc.

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 non-consanguineous. 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

© 2013 Wiley Periodicals, Inc.

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.

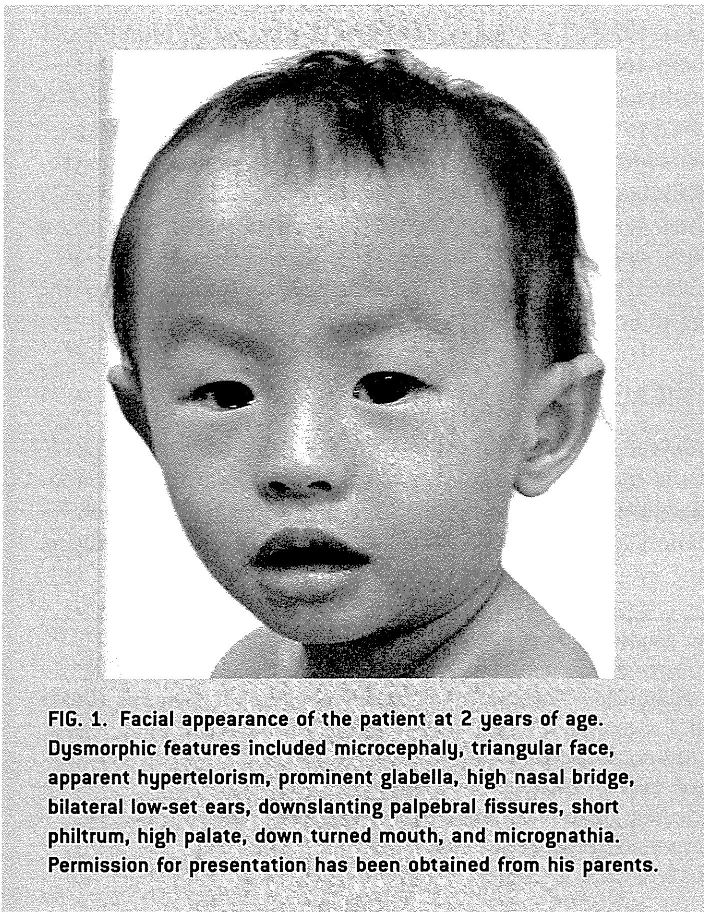

FIG. 1. Facial appearance of the patient at 2 years of age. Dysmorphic features included microcephaly, triangular face, apparent hypertelorism, prominent glabella, high nasal bridge, bilateral low-set ears, downslanting palpebral fissures, short philtrum, high palate, down turned mouth, and micrognathia.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

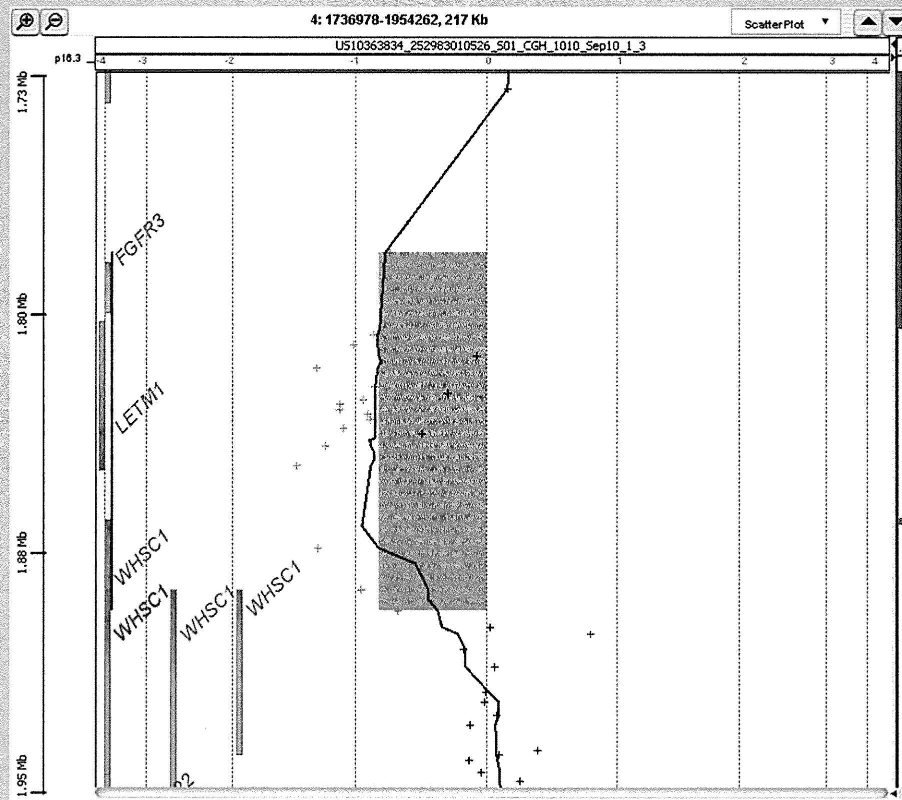

FIG. 2. Array-CGH revealed loss of genomic copy numbers in the region 4p16.3, which included the *FGFR3*, *LETM1*, and 5′ end of *WHSC1*. Blue rectangle indicates the region of 109 kb copy loss. Major transcript variants of *WHSC1* are shown by four bars. The deletion involved 5′ part of the major *WHSC1*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

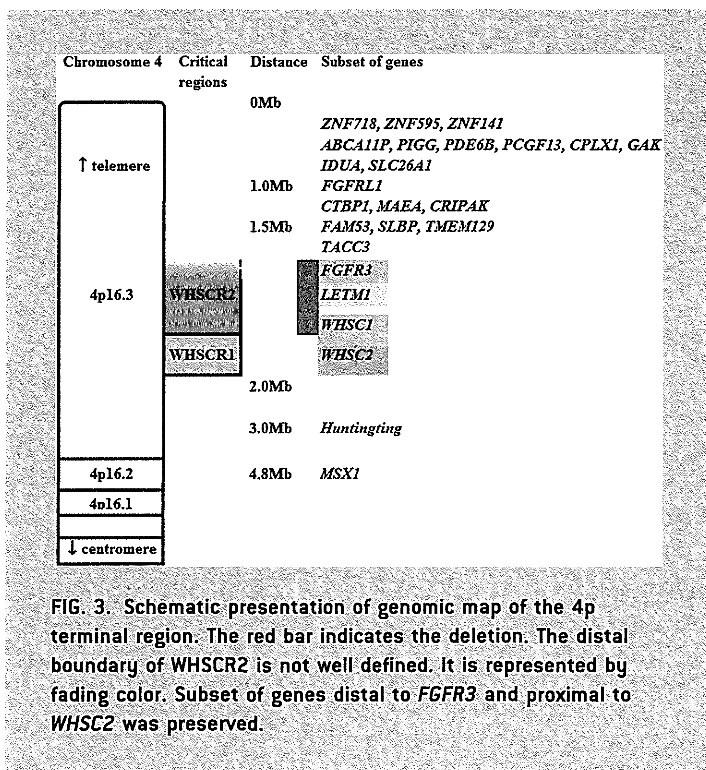

FIG. 3. Schematic presentation of genomic map of the 4p terminal region. The red bar indicates the deletion. The distal boundary of WHSCR2 is not well defined. It is represented by fading color. Subset of genes distal to *FGFR3* and proximal to *WHSC2* was preserved.

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*.

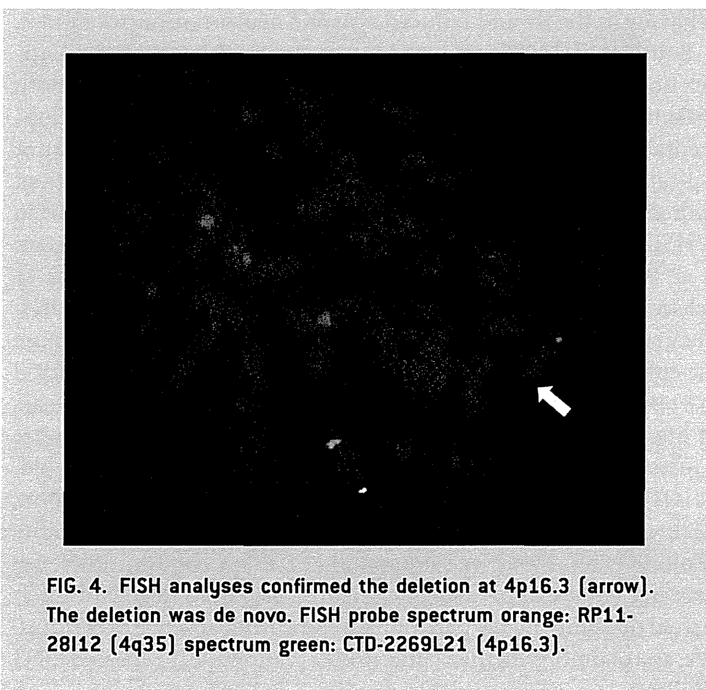

FIG. 4. FISH analyses confirmed the deletion at 4p16.3 (arrow). The deletion was de novo. FISH probe spectrum orange: RP11-28I12 (4q35) spectrum green: CTD-2269L21 (4p16.3).

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.

CLINICAL REPORT

AMERICAN JOURNAL OF medical genetics PART A

1p34.3 Deletion Involving *GRIK3*: Further Clinical Implication of GRIK Family Glutamate Receptors in the Pathogenesis of Developmental Delay

Toshiki Takenouchi,[1] Noriko Hashida,[1] Chiharu Torii,[2] Rika Kosaki,[3] Takao Takahashi,[1] and Kenjiro Kosaki[2]*

[1]Department of Pediatrics, Keio University School of Medicine, Tokyo, Japan

[2]Center for Medical Genetics, Keio University School of Medicine, Tokyo, Japan

[3]Division of Medical Genetics, National Center for Child Health and Development, Tokyo, Japan

Manuscript Received: 20 May 2013; Manuscript Accepted: 25 August 2013

A growing body of evidence suggests an association between microdeletion/microduplication and schizophrenia/intellectual disability. Abnormal neurogenesis and neurotransmission have been implicated in the pathogenesis of these neuropsychiatric and neurodevelopmental disorders. The kainate/AMPA-type ionotropic glutamate receptor (GRIK = glutamate receptor, ionotropic, kainate) plays a critical role in synaptic potentiation, which is an essential process for learning and memory. Among the five known GRIK family members, haploinsufficiency of *GRIK1*, *GRIK2*, and *GRIK4* are known to cause developmental delay, whereas the roles of *GRIK3* and *GRIK5* remain unknown. Herein, we report on a girl who presented with a severe developmental delay predominantly affecting her language and fine motor skills. She had a 2.6-Mb microdeletion in 1p34.3 involving *GRIK3*, which encodes a principal subunit of the kainate-type ionotropic glutamate receptor. Given its strong expression pattern in the central nervous system and the biological function of GRIK3 in presynaptic neurotransmission, the haploinsufficiency of *GRIK3* is likely to be responsible for the severe developmental delay in the proposita. A review of genetic alterations and the phenotypic effects of all the GRIK family members support this hypothesis. The current observation of a microdeletion involving *GRIK3*, a kainate-type ionotropic glutamate receptor subunit, and the neurodevelopmental manifestation in the absence of major dysmorphism provides further clinical implication of the possible role of GRIK family glutamate receptors in the pathogenesis of developmental delay.

© 2013 Wiley Periodicals, Inc.

Key words: GRIK3; glutamate receptor; developmental delay

How to Cite this Article:
Takenouchi T, Hashida N, Torii C, Kosaki R, Takahashi T, Kosaki K. 2014. 1p34.3 Deletion involving *GRIK3*: Further clinical implication of GRIK family glutamate receptors in the pathogenesis of developmental delay.

Am J Med Genet Part A 164A:456–460.

INTRODUCTION

An emerging paradigm suggests that intellectual disability and schizophrenia are associated with microdeletion/microduplication at several specific genetic loci [Crespi et al., 2010; Malhotra and Sebat, 2012]. This association is best exemplified by the chromosome locus 16p11.2, where microdeletion/microduplication are associated with autism and schizophrenia [Weiss et al., 2008; McCarthy et al., 2009]. These studies have led to the identification of a candidate gene (*KTCD13*) that has been implicated in the cell cycle during neurogenesis [Golzio et al., 2012].

Along with abnormal neurogenesis, the pathogenesis of major neurodevelopmental and neuropsychiatric illnesses involves aberrant neurotransmission. Among the major neurotransmitters, glutaminergic and GABAergic systems play critical roles [Stawski et al., 2010; Niciu et al., 2012]. Both metabotropic and ionotropic types of glutamate receptors are known to exist: the latter has been subclassified into NMDA (*N*-methyl-D-aspartate), and kainate/

Conflict of interest: none.
Grant sponsor: Research on Applying Health Technology; Grant number: H23-013; Grant sponsor: Ministry of Health, Labour and Welfare, Japan.
Abbreviations: CGH, comparative genome hybridization; GRIK, glutamate receptor, ionotropic, kainate.
*Correspondence to:
Kenjiro Kosaki, M.D., Center for Medical Genetics, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan.
E-mail: kkosaki@z3.keio.jp
Article first published online in Wiley Online Library (wileyonlinelibrary.com): 25 November 2013
DOI 10.1002/ajmg.a.36240

AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole-4-propionic acid) receptors [Egebjerg et al., 1991]. The kainate/AMPA receptor is widely expressed in the central nervous system and is a key molecule in synaptic plasticity, an essential process for learning and memory [Bortolotto et al., 1999; Schmitz et al., 2001].

The kainate receptor (GRIK = glutamate receptor, ionotropic, kainate) is a tetrameric receptor composed of three principal subunits, GRIK1-3 (formerly GluR5-7), and two auxiliary subunits, GRIK4-5 (formerly KA1-2)[Fernandes et al., 2009]. Compared with other kainate/AMPA receptors, the GRIK3-containing kainate receptor has an atypical electrophysiological function: it has a very low affinity to kainate/glutamate because of fast desensitization [Schiffer et al., 1997; Perrais et al., 2009]. Indeed, *Grik3*$^{-/-}$ mice exhibit markedly impaired short- and long-term synaptic potentiation [Pinheiro et al., 2007]. This discrepant electrophysiological property in GRIK3 suggests that the presence of other subunits cannot compensate for defective GRIK3.

Several nucleotide polymorphisms in *GRIK3* are known to be associated with major neuropsychiatric illnesses, such as schizophrenia and recurrent major depressive disorder [Begni et al., 2002; Schiffer and Heinemann, 2007; Kilic et al., 2010]. However, the phenotypic effect of *GRIK3* deletion remains to be elucidated.

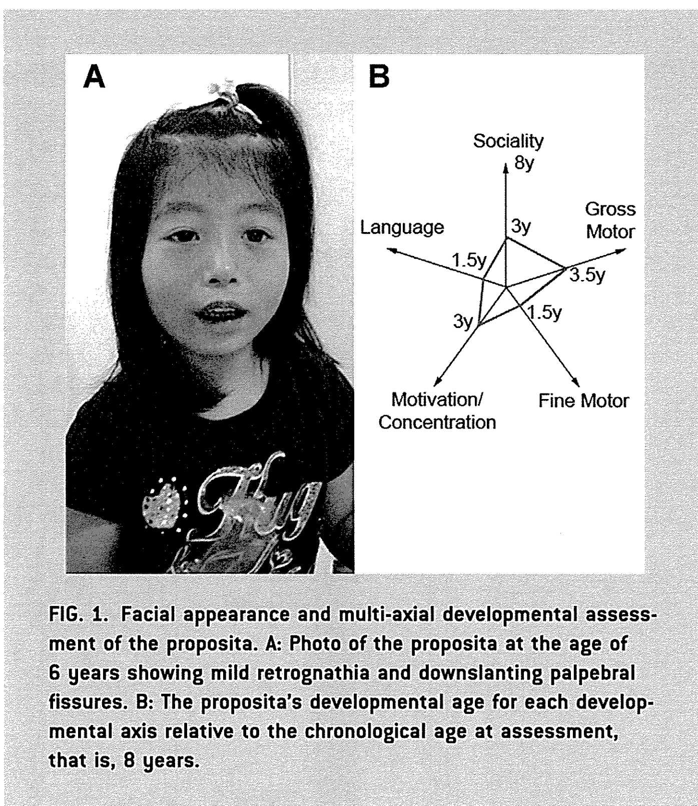

FIG. 1. Facial appearance and multi-axial developmental assessment of the proposita. A: Photo of the proposita at the age of 6 years showing mild retrognathia and downslanting palpebral fissures. B: The proposita's developmental age for each developmental axis relative to the chronological age at assessment, that is, 8 years.

CLINICAL REPORT

The proposita was an 8-year-old Japanese girl born to nonconsanguineous parents. She was born at 37 weeks of gestation with a birth weight of 1,818 g (−3.0 SD) and a length of 44.5 cm (−1.9 SD). She exhibited poor weight gain during infancy and was fed via tube feeding until the age of 5 years. In addition to her failure to thrive, she exhibited severe delays in psychomotor development. She gained head control at the age of 8 months, and she walked independently at the age of 2½ years. A physical examination showed no focal neurological deficits, major physical deformities, or dysmorphisms except for mild retrognathia and slightly downslanting palpebral fissures (Fig. 1A).

At the age of 8 years, an experienced child psychologist blindly evaluated her developmental status for multiple axes, based on the Japanese standard developmental scales and a parental interview. This evaluation demonstrated marked impairment predominantly in her language and fine motor skills, compared with her gross motor skills and sociality (Fig. 1B).

Gross Motor Development

She was able to run, but she was unable to stand on one foot. She could throw a ball, but she could not catch one. Her gross motor skills were equivalent to an age of 3.5 years.

Fine Motor Development

She was able to pinch objects, but she was unable to stack blocks. She was barely able to complete a simple puzzle. She could scribble, but she could not draw lines. Overall, her fine motor skills were equivalent to an age of approximately 1.5 years.

Language Development

She was able to speak four words: "bye-bye," "dada," "mama," and "good." She did not point to objects, but she chose objects and pictures that she wanted. She understood a few words using sign language. Her language development was equivalent to 1.5 years.

Social Development

She was able to drink from a cup and to wash her hands without assistance. She could eat with a spoon and a fork but required significant assistance. She required help to brush her teeth and to change her clothes. Her social development was equivalent to approximately 3 years of age.

Motivation/Concentration

She lacked the necessary concentration and motivation to complete tasks. We considered her attention span/concentration as being equivalent to approximately 3 years of age.

MOLECULAR ANALYSIS

A microarray analysis using an array CGH platform (ISCA 4 × 180k; Agilent Technologies, CA) revealed a de novo 2.6-Mb deletion in 1p34.3, extending from position 34,632,258–37,241,519 (NCBI36/hg18, March 2006). The deleted interval included 44 genes (Fig. 2).

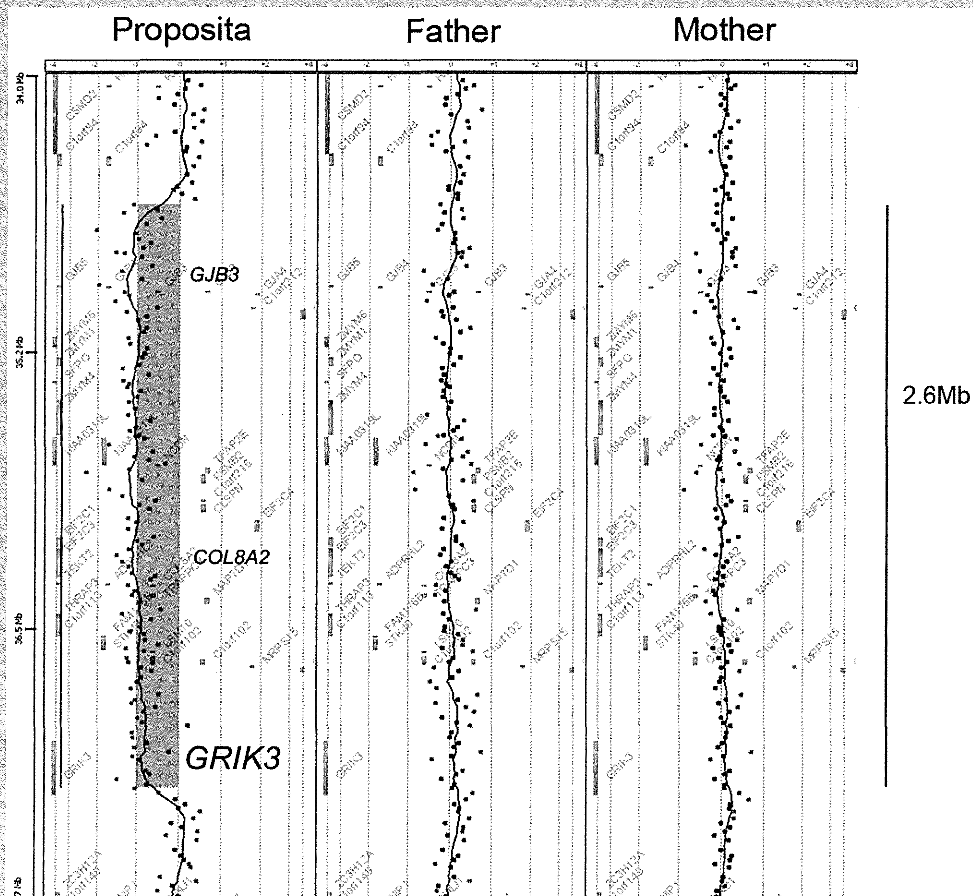

FIG. 2. Microarray analyses of the proposita and her parents Note that the proposita (left) had a 2.6-Mb deletion (highlighted in blue). The deleted interval included *GRIK3*, *COL8A2* and *GJB3*. Neither her father (middle) nor her mother (right) had the same microdeletion, confirming that the microdeletion in the proposita was de novo.

DISCUSSION

A young girl with a 1p34.3 microdeletion manifested a severe developmental delay without any major recognizable dysmorphism. The 2.6-Mb microdeletion included *GRIK3*, which encodes a subunit of a kainate-type ionotropic glutamate receptor that is strongly expressed in the central nervous system [Bettler et al., 1992].

From the standpoint of the 1p34.3 microdeletion, two candidate genes for developmental delay have been previously reported. A boy with autistic spectrum disorder who had a de novo 3.3-Mb microdeletion in 1p34.2p34.3 was reported, and his autistic phenotype was attributed to *RIMS3* (OMIM 611600)[Kumar et al., 2010]. Another boy with autistic spectrum disorder had a 4.1-Mb microdeletion in 1p34.2–34.3 and exhibited a severe developmental delay, microcephaly, and facial dysmorphism. *SLC2A1* (OMIM 138140) was considered a candidate gene for his developmental delay [Vermeer et al., 2007]. Since neither *RIMS3* nor *SLC2A1* was included in the deleted interval in the proposita, the mechanistic basis for the severe developmental delay was thought to differ.

Among the genes located in the deleted interval in the proposita, the biological function of the *GRIK3* gene made it the most plausible candidate for the severe developmental delay. The developmental characteristics of the proposita, which included significant deficits in language with spared gross motor function, were not incompatible with aberrant glutamate neurotransmission. This reasoning was also supported by observations in *Grik3*$^{-/-}$ mice, as these mice exhibit impaired synaptic transmission [Pinheiro et al., 2007].

We reviewed the genetic alterations of the kainate receptor family subunits, GRIK1-5, and their neuropsychiatric and neurodevelopmental manifestations (Table I). Associations between polymorphisms in each GRIK family member and major neuropsychiatric illnesses, such as schizophrenia and bipolar disorders, have been repeatedly demonstrated by multiple groups [Begni et al., 2002; Jamain et al., 2002; Pickard et al., 2006; Schiffer and Heinemann, 2007; Kilic et al., 2010; Sampaio et al., 2011; Yosifova et al., 2011; Hirata et al., 2012]. Patients with deletions of GRIK family genes and non-syndromic intellectual disability have also been reported by several authors [Pickard et al., 2006; Motazacker et al., 2007; Bonaglia et al., 2008; Haldeman-Englert et al., 2010].

TABLE I. Phenotypic Effect of Genetic Alterations of All the Kainate Receptor Subunits (GRIK Family)

Gene name and chromosomal locus	Polymorphism and association studies with psychiatric disorders	Deletion with developmental delay/intellectual disability
GRIK1 at 21q21.3	SCZ [Hirata et al., 2012]	ID [Haldeman-Englert et al., 2010]
GRIK2 at 6q16.3	ASD [Jamain et al., 2002]	ID [Motazacker et al., 2007]
	OCD [Sampaio et al., 2011]	ID/PWS-like [Bonaglia et al., 2008]
GRIK3 at 1p34.3	SCZ [Begni et al., 2002; Kilic et al., 2010]	The present report
	MDD [Schiffer and Heinemann, 2007]	
GRIK4 at 11q23.3	SCZ, BPD [Pickard et al., 2006]	ID [Pickard et al., 2006]
GRIK5 at 19q13.2	BPD [Yosifova et al., 2011]	NR

ASD, autisic spectrum disorder; BPD, bipolar disorder; ID, intellectual disability; MDD, major depressive disorder; NR, not reported; PWS, Prader–Willi-syndrome; SCZ, Schizophrenia.

The present report provides an additional piece of missing information regarding the neurobehavioral effects of genetic alterations in GRIK family genes, namely, the effect of a *GRIK3* deletion. It is possible that *GRIK3* represents the causative locus at 1p34 that has been implicated in a linkage study of schizophrenia [DeLisi et al., 2002] and in a genome-wide transcriptome analysis of autism spectrum disorders [Luo et al., 2012].

From a clinical standpoint, it is notable that while attempting to determine the etiology of the developmental delay, we were incidentally able to detect two causative genes for autosomal dominant late-onset diseases in the microarray analysis. Heterozygous and digenic mutations in *GJB3* and *COL8A2* could lead to autosomal dominant deafness 2B (OMIM 612644) [Xia et al., 1998; Liu et al., 2009] and two types of corneal dystrophy, that is, polymorphous posterior corneal dystrophy (OMIM 609140) and Fuchs endothelial corneal dystrophy (OMIM 136800) [Biswas et al., 2001], respectively. Since most causative mutations in *GJB3* and *COL8A2* are missense mutations, not truncating mutations, the mechanistic basis of these entities may be dominant-negative mutations, rather than haploinsufficiency. If so, the deletions of *GJB3* and *COL8A2* might not be clinically relevant.

In conclusion, we document a young girl with a microdeletion involving *GRIK3*, a kainate-type ionotropic glutamate receptor subunit. This predominant neurodevelopmental manifestation in the absence of major dysmorphism provides further clinical implication of the role of GRIK family glutamate receptors in the pathogenesis of developmental delay.

ACKNOWLEDGMENT

We thank Namiko Saito and Yumi Obayashi for their technical assistance in article preparation.

REFERENCES

Begni S, Popoli M, Moraschi S, Bignotti S, Tura GB, Gennarelli M. 2002. Association between the ionotropic glutamate receptor kainate 3 (GRIK3) ser310ala polymorphism and schizophrenia. Mol Psychiatry 7:416–418.

Bettler B, Egebjerg J, Sharma G, Pecht G, Hermans-Borgmeyer I, Moll C, Stevens CF, Heinemann S. 1992. Cloning of a putative glutamate receptor: A low affinity kainate-binding subunit. Neuron 8:257–265.

Biswas S, Munier FL, Yardley J, Hart-Holden N, Perveen R, Cousin P, Sutphin JE, Noble B, Batterbury M, Kielty C, Hackett A, Bonshek R, Ridgway A, McLeod D, Sheffield VC, Stone EM, Schorderet DF, Black GC. 2001. Missense mutations in COL8A2, the gene encoding the alpha2 chain of type VIII collagen, cause two forms of corneal endothelial dystrophy. Hum Mol Genet 10:2415–2423.

Bonaglia MC, Ciccone R, Gimelli G, Gimelli S, Marelli S, Verheij J, Giorda R, Grasso R, Borgatti R, Pagone F, Rodriguez L, Martinez-Frias ML, van Ravenswaaij C, Zuffardi O. 2008. Detailed phenotype-genotype study in five patients with chromosome 6q16 deletion: Narrowing the critical region for Prader–Willi-like phenotype. Eur J Hum Genet 16:1443–1449.

Bortolotto ZA, Clarke VR, Delany CM, Parry MC, Smolders I, Vignes M, Ho KH, Miu P, Brinton BT, Fantaske R, Ogden A, Gates M, Ornstein PL, Lodge D, Bleakman D, Collingridge GL. 1999. Kainate receptors are involved in synaptic plasticity. Nature 402:297–301.

Crespi B, Stead P, Elliot M. 2010. Evolution in health and medicine Sackler colloquium: Comparative genomics of autism and schizophrenia. Proc Natl Acad Sci USA 107:1736–1741.

DeLisi LE, Mesen A, Rodriguez C, Bertheau A, LaPrade B, Llach M, Riondet S, Razi K, Relja M, Byerley W, Sherrington R. 2002. Genome-wide scan for linkage to schizophrenia in a Spanish-origin cohort from Costa Rica. Am J Med Genet 114:497–508.

Egebjerg J, Bettler B, Hermans-Borgmeyer I, Heinemann S. 1991. Cloning of a cDNA for a glutamate receptor subunit activated by kainate but not AMPA. Nature 351:745–748.

Fernandes HB, Catches JS, Petralia RS, Copits BA, Xu J, Russell TA, Swanson GT, Contractor A. 2009. High-affinity kainate receptor subunits are necessary for ionotropic but not metabotropic signaling. Neuron 63:818–829.

Golzio C, Willer J, Talkowski ME, Oh EC, Taniguchi Y, Jacquemont S, Reymond A, Sun M, Sawa A, Gusella JF, Kamiya A, Beckmann JS, Katsanis N. 2012. KCTD13 is a major driver of mirrored neuroanatomical phenotypes of the 16p11.2 copy number variant. Nature 485:363–367.

Haldeman-Englert CR, Chapman KA, Kruger H, Geiger EA, McDonald-McGinn DM, Rappaport E, Zackai EH, Spinner NB, Shaikh TH. 2010. A de novo 8.8-Mb deletion of 21q21.1-q21.3 in an autistic male with a complex rearrangement involving chromosomes 6, 10 and 21. Am J Med Genet A 152A:196–202.

Hirata Y, Zai CC, Souza RP, Lieberman JA, Meltzer HY, Kennedy JL. 2012. Association study of GRIK1 gene polymorphisms in schizophrenia: case-control and family-based studies. Hum Psychopharmacol 27:345–351.

Jamain S, Betancur C, Quach H, Philippe A, Fellous M, Giros B, Gillberg C, Leboyer M, Bourgeron T. Paris Autism Research International Sibpair S. 2002. Linkage and association of the glutamate receptor 6 gene with autism. Mol Psychiatry 7:302–310.