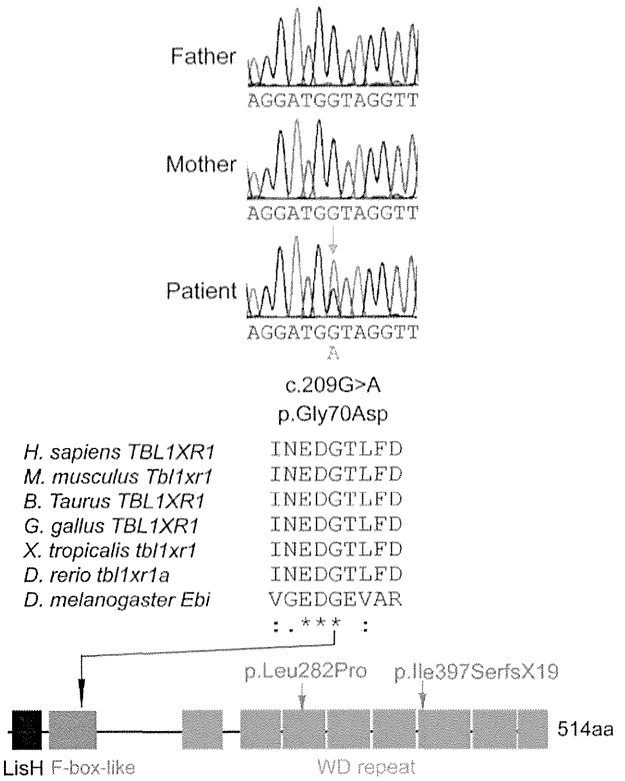

Figure 2 *De novo TBL1XR1* mutation. The c.209G>A (p.Gly70Asp) mutation occurred *de novo* at an evolutionarily conserved amino acid in an F-box-like domain. Multiple amino-acid sequences of TBL1XR1 proteins were aligned with tools available on the CLUSTALW web site. Two previously reported mutations (p.Leu282Pro and p.Ile397SefsX19) are highlighted in red. A full color version of this figure is available at the *Journal of Human Genetics* journal online.

575 in-house control exomes. Both mutations were predicted as damaging by SIFT and PolyPhen2. However, MutationTaster classified only p.Gly70Asp in *TBL1XR1* as damaging whereas p.Thr265Met in *SELPLG* was predicted as a polymorphism. We found no recessive mutations in known early-onset epileptic encephalopathy genes including *SLC25A22*, *PNPO*, *PNKP* and *PLCB1*.[7] All experimental protocols used were approved by the Institutional Review Board of Yokohama City University School of Medicine.

SELPLG encodes P-selectin glycoprotein ligand 1, for which a knock-out mouse study showed that *Selplg* is required for leukocyte adhesion and rolling.[8,9] No neurological abnormalities were reported, suggesting that the *SELPLG* mutation is less likely to be involved in the phenotype of this patient.

TBL1XR1, also denoted as *TBLR1*, is required for β-catenin–Tcf-mediated Wnt signaling.[1,2] Mutations in *TCF4*, an essential mediator of Wnt signaling, have been shown to cause Pitt–Hopkins Syndrome, which presents with severe intellectual disability, seizures and stereotypic movements.[10,11] This suggests that the β-catenin–Tcf-mediated Wnt pathway of signaling is essential for normal brain function. Moreover, two *de novo TBL1XR1* mutations (p.Leu282Pro and p.Ile397SerfsX19) were found in 2 of 2446 patients with autism spectrum disorders.[4,5] In our case, the p.Gly70Asp mutation occurred in an evolutionarily conserved amino acid within an F-box-like domain (Figure 2). Indeed, the F-box-like domain of TBLR1 (TBL1XR1) is essential for a high affinity interaction between TBL1XR1 and SMRT, a co-repressor of nuclear hormone receptors.[3] This implies that p.Gly70Asp may affect this interaction. Therefore, the evidence suggests that the p.Gly70Asp mutation may cause a West syndrome phenotype with Rett-like and autistic features.

The role of *TBL1XR1* mutations was also investigated in 280 epileptic patients. High-resolution melting analysis revealed that three rare missense variants (p.Ala116Ser, p.Gly405Glu and p.Asn407Ser) were present in three patients. Since they were predicted as benign by PolyPhen-2, these mutations are unlikely to be pathogenic. These data suggest that *TBL1XR1* mutations are rarely involved in epileptic patients.

In conclusion, we describe a Japanese girl with a *de novo TBL1XR1* mutation that is predicted as pathogenic. Our report suggests that the clinical spectrum of *TBL1XR1* mutations includes autistic features as a core phenotype, as well as presenting with West syndrome and Rett-like features.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

We would like to thank the patient and her family for their participation in this study. We thank Kiyomi Nishiyama for her technical assistance. This study was supported by: the Japanese Ministry of Health, Labour, and Welfare; the Japan Society for the Promotion of Science (a Grant-in-Aid for Scientific Research (B) (25293085, 25293235) and a Grant-in-Aid for Scientific Research (A) (13313587)); the Takeda Science Foundation; the fund for Creation of Innovation Centers for Advanced Interdisciplinary Research Areas Program in the Project for Developing Innovation Systems; the Strategic Research Program for Brain Sciences (11105137); and a Grant-in-Aid for Scientific Research on Innovative Areas (Transcription Cycle) from the Japanese Ministry of Education, Culture, Sports, Science, and Technology (12024421).

1 Li, J. & Wang, C. Y. TBL1-TBLR1 and beta-catenin recruit each other to Wnt target-gene promoter for transcription activation and oncogenesis. *Nat. Cell Biol.* **10,** 160–169 (2008).
2 Choi, H. K., Choi, K. C., Yoo, J. Y., Song, M., Ko, S. J., Kim, C. H. *et al.* Reversible SUMOylation of TBL1-TBLR1 regulates beta-catenin-mediated Wnt signaling. *Mol. Cell* **43,** 203–216 (2011).
3 Zhang, X. M., Chang, Q., Zeng, L., Gu, J., Brown, S. & Basch, R. S. TBLR1 regulates the expression of nuclear hormone receptor co-repressors. *BMC Cell Biol.* **7,** 31 (2006).
4 O'Roak, B. J., Vives, L., Girirajan, S., Karakoc, E., Krumm, N., Coe, B. P. *et al.* Sporadic autism exomes reveal a highly interconnected protein network of de novo mutations. *Nature* **485,** 246–250 (2012).
5 O'Roak, B. J., Vives, L., Fu, W., Egertson, J. D., Stanaway, I. B., Phelps, I. G. *et al.* Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. *Science* **338,** 1619–1622 (2012).
6 Walsh, T., Lee, M. K., Casadei, S., Thornton, A. M., Stray, S. M., Pennil, C. *et al.* Detection of inherited mutations for breast and ovarian cancer using genomic capture and massively parallel sequencing. *Proc. Natl Acad. Sci. USA* **107,** 12629–12633 (2010).
7 Mastrangelo, M. & Leuzzi, V. Genes of early-onset epileptic encephalopathies: from genotype to phenotype. *Pediatr. Neurol.* **46,** 24–31 (2012).
8 Yang, J., Hirata, T., Croce, K., Merrill-Skoloff, G., Tchernychev, B., Williams, E. *et al.* Targeted gene disruption demonstrates that P-selectin glycoprotein ligand 1 (PSGL-1) is required for P-selectin-mediated but not E-selectin-mediated neutrophil rolling and migration. *J. Exp. Med.* **190,** 1769–1782 (1999).
9 Xia, L., Sperandio, M., Yago, T., McDaniel, J. M., Cummings, R. D., Pearson-White, S. *et al.* P-selectin glycoprotein ligand-1-deficient mice have impaired leukocyte tethering to E-selectin under flow. *J. Clin. Invest.* **109,** 939–950 (2002).
10 Zweier, C., Peippo, M. M., Hoyer, J., Sousa, S., Bottani, A., Clayton-Smith, J. *et al.* Haploinsufficiency of TCF4 causes syndromal mental retardation with intermittent hyperventilation (Pitt-Hopkins syndrome). *Am. J. Hum. Genet.* **80,** 994–1001 (2007).
11 Amiel, J., Rio, M., de Pontual, L., Redon, R., Malan, V., Boddaert, N. *et al.* Mutations in TCF4, encoding a class I basic helix-loop-helix transcription factor, are responsible for Pitt-Hopkins syndrome, a severe epileptic encephalopathy associated with autonomic dysfunction. *Am. J. Hum. Genet.* **80,** 988–993 (2007).

CLINICAL GENETICS An International Journal of Genetics, Molecular and Personalized Medicine

Clin Genet 2014
Printed in Singapore. All rights reserved

CLINICAL GENETICS
doi: 10.1111/cge.12492

Short Report

Targeted next-generation sequencing in the diagnosis of neurodevelopmental disorders

Okamoto N., Miya F., Tsunoda T., Kato M., Saitoh S., Yamasaki M., Shimizu A., Torii C., Kanemura Y., Kosaki K.. Targeted next-generation sequencing in the diagnosis of neurodevelopmental disorders.
Clin Genet 2014. © John Wiley & Sons A/S. Published by John Wiley & Sons Ltd, 2014

We developed a next-generation sequencing (NGS) based mutation screening strategy for neurodevelopmental diseases. Using this system, we screened 284 genes in 40 patients. Several novel mutations were discovered. Patient 1 had a novel mutation in *ACTB*. Her dysmorphic feature was mild for Baraitser-Winter syndrome. Patient 2 had a truncating mutation of *DYRK1A*. She lacked microcephaly, which was previously assumed to be a constant feature of *DYRK1A* loss of function. Patient 3 had a novel mutation in *GABRD* gene. She showed Rett syndrome like features. Patient 4 was diagnosed with Noonan syndrome with *PTPN11* mutation. He showed complete agenesis of corpus callosum. We have discussed these novel findings.

Conflict of interest

The authors report no conflicts of interest.

N. Okamoto[a], F. Miya[b], T. Tsunoda[b], M. Kato[c], S. Saitoh[d], M. Yamasaki[e], A. Shimizu[f], C. Torii[g], Y. Kanemura[h,i] and K. Kosaki[g]

[a]Department of Medical Genetics, Osaka Medical Center and Research Institute for Maternal and Child Health, Osaka, Japan, [b]Laboratory for Medical Science Mathematics, Center for Integrative Medical Sciences, RIKEN, Yokohama, Japan, [c]Department of Pediatrics, Yamagata University Faculty of Medicine, Yamagata, Japan, [d]Department of Pediatrics and Neonatology, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan, [e]Department of Pediatric Neurosurgery, Takatsuki General Hospital, Osaka, Japan, [f]Division of Biomedical Information Analysis, Iwate Tohoku Medical Megabank Organization, Iwate Medical University, Iwate, Japan, [g]Center for Medical Genetics, Keio University School of Medicine, Tokyo, Japan, [h]Division of Regenerative Medicine, Institute for Clinical Research, Osaka National Hospital, National Hospital Organization, Osaka, Japan, and [i]Department of Neurosurgery, Osaka National Hospital, National Hospital Organization, Osaka, Japan

Key words: Baraitser-Winter syndrome – *DYRK1A* – *GABRD* – next-generation sequencing

Corresponding author: 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.
Tel.: +81 725 56 1220;
fax: +81 725 56 5682;
e-mail: okamoto@osaka.email.ne.jp

Received 26 April 2014, revised and accepted for publication 19 August 2014

Despite many recent studies focusing on discovering the genetic basis of neurodevelopmental diseases, it is still largely unknown. We developed a next-generation sequencing (NGS) based mutation screening strategy. We screened 284 genes known or predicted to be associated with neurodevelopmental disorders with microcephaly/macrocephaly, central nervous system (CNS) anomalies and intellectual disability (ID).

Materials and methods

We studied 40 patients with neurodevelopmental disorders. They were negative for conventional cytogenetic studies and microarray analysis. With the approval of our institutional ethics committee, the patients were analyzed using this targeted sequencing. The genomic DNA of each patient was extracted from peripheral blood using extraction kit. Detail of the cell sample preparation was described in Supporting information.

Target gene sequencing

Three microgram of each sample DNA was sheared to 150–200 bp using the Covaris DNA Shearing System (Wobum, MA, USA). To capture the target exonic DNA, we used the SureSelectXT Custom capture library (Agilent, Santa Clara, CA) for 1.6 Mb of exons of neuronal gene capture. The sequence library was constructed with the SureSelect XT Target Enrichment System for Illumina Paired-End Sequencing Library kit (Agilent) according to the manufacturer's instructions. We performed DNA sequencing of either 76- or 101-bp paired-end reads using the Illumina Genome Analyzer IIx (Illumina, San Diego, CA) and HiSeq 2000 sequencer (Illumina, San Diego, CA).

Single nucleotide variation (SNV) calling

NGS reads were aligned to the Human reference genome (GRCh37/hg19). We then excluded polymerase chain reaction (PCR) duplicates, and extracted reads uniquely mapped to the reference genome that were properly paired within the insert size within mean ± 2 standard deviation (SD) of the mean. Base calling was performed in on-target regions, those regions within 100 bp upstream and downstream of the exon capture probes. SNV and insertion and deletion (indel) calling were performed using SAM TOOLS and GATK software. We excluded known variants found in database. We then narrowed the candidates to only non-synonymous, nonsense and splice site SNVs and frame shift indels. More details of method for variant calling are described in Supporting information.

NGS base-call quality check

To analyze the quality of our base-calling algorithm, we used genotypes from HapMap database (release #28, obtained from ftp://ftp.ncbi.nlm.nih.gov/hapmap/genotypes/2010-08_phaseII+III/). Sanger sequence validation of SNVs was performed using Applied Biosystems 3730xl DNA Analyzer (Life Technologies, Carlsbad, CA).

Results

To identify the causal mutation for neuronal diseases, we designed custom capture probes for the exons of 284 neuronal genes (Table S1, Supporting information). We performed targeted genes sequencing using these probes and generated 1.7 Gb of sequence on average. The average read depth of the on-target regions was 608. To check the quality of our NGS base calls, we sequenced HapMap-JPT NA18943 using the same method as the other samples, and compared our NGS calls with the released genotype of the HapMap consortium. The genotypes for 3129 locations were comparable between the two data sets. All but 16 of the 3129 genotypes were concordant between our NGS calls and the HapMap data. We validated these mismatched 16 positions using Sanger sequencing and all 16 were consistent with our NGS calls (Tables S2 and S3). On the basis of this, we estimate the false positive and false negative rate of our SNV calling to be <0.032% (<1/3129).

Clinical reports

In all patients, developmental quotient (DQ) was measured using the Kyoto Scale of Psychological Development test.

Patient 1 with *ACTB* mutation

The 3-year-old female was born at 37 weeks of gestation by normal delivery. Her developmental milestones were markedly delayed. She sat unsupported at 18 months of age. Recently, she walked with support. She spoke several meaningful words. Her DQ was 39 at 2 years of age. Physical examination identified dysmorphic features, including a flat face, arched eyebrows, narrow palpebral fissures, low-set posteriorly rotated ears and a thin upper lip. Ophthalmological investigation revealed no colobomata. Her height was 86.3 cm (−0.8 SD), and weight was 12.3 kg (mean). Her head circumference was 50 cm (+1.2 SD) at 2 years and 6 months of age. Neuroradiological investigations revealed enlarged lateral ventricles, decreased white matter volume and pachygyria dominant in the frontal lobe (Fig. 1a,b).

A novel missense was identified in *ACTB*, c.733G>A, p.G245S. She was therefore diagnosed with Baraitser-Winter syndrome (BRWS) (1).

Patient 2 with *DYRK1A* mutation

The 7-year-old female patient was born at 39 weeks of gestation by induced delivery. Her developmental milestones were severely retarded. She could not walk independently. She had no communicative language. In addition, her visual acuity was disturbed by severe amblyopia. She could see and reach objects within 30 cm. She exhibited self-injurious behavior, temper tantrums and vocal tics by vibrating her palate. She

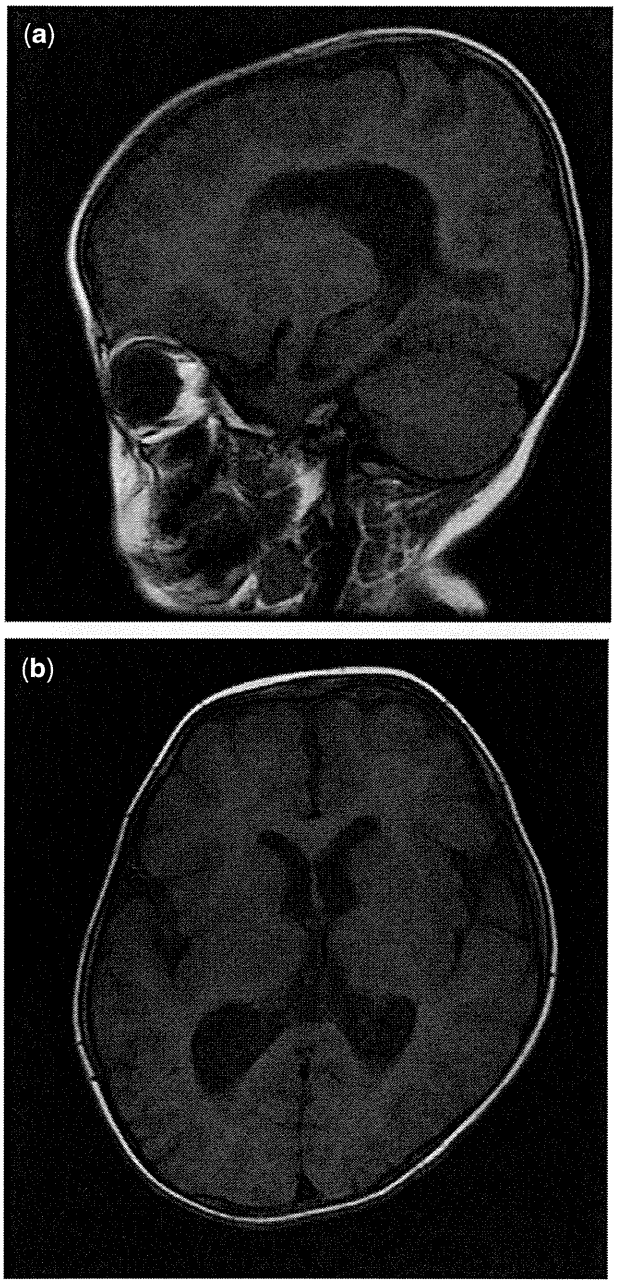

Fig. 1. (**a**, **b**) T1-weighted magnetic resonance image (MR) of patient 1 with *ACTB* mutation shows enlarged lateral ventricles, decreased white matter volume and pachygyria dominant in the frontal lobe.

was diagnosed with autism spectrum disorder (ASD) according to DSM-5. Her DQ was not properly assessed because of visual disturbance.

Physical examination identified dysmorphic features, including frontal bossing, hypertelorism, nystagmus, epicanthal folds, a flat nasal bridge, bilateral low-set ears, down-slanting palpebral fissures, a short philtrum, a high arched palate, downturned mouth and micrognathia. Her weight was 14.6 kg (−2.2 SD), height was 103.5 cm (−3.1 SD) and head circumference was 52 cm (+0.6 SD). She showed relative macrocephaly.

Brain computerized tomography (CT) and magnetic resonance imaging (MRI) were normal. Retinal abnormalities and optic nerve hypoplasia were not identified by fundoscopic investigations. Electroencephalography (EEG) showed no epileptic discharges. She had an early termination codon in exon 11 of the *DYRK1A* gene (c.C1699T: p.Q567*).

Patient 3 with *GABRD* mutation

The 12-year-old female was born at 41 weeks of gestation by induced delivery. Her development was severely retarded with generalized muscular hypotonia. She sat alone at 4 years of age. She cannot walk independently. She spoke no meaningful words. Her DQ was 12 at 9 years of age. She showed stereotyped behavior including hand gripping and bruxism. Purposeful hand skills were not obtained. She was diagnosed with Rett syndrome. EEG revealed bilateral occipital dominant high voltage slow spike and wave complex. Her height was 137 cm (−3.4 SD), weight was 35 kg (−2.1 SD) and head circumference was 51 cm (−1.8 SD). Brain CT and MRI were normal.

She had 2 bp insertion–deletion corresponding to two amino acids in *GABRD* gene (c.G498A:p.M166I and, c.G499A: p.D167N) (Fig. 2). This mutation was *de novo*.

Patient 4 with *PTPN11* mutation

The 4-year-old male was born at 40 weeks of gestation by normal delivery. Profound sensorineural hearing loss was confirmed. He was able to control his head at 4 months, roll over at 6 months of age. He could sit without support at 14 months of age. He started to walk without support at 3 years of age. His height was 90.7 cm (−1.8 SD), weight was 14.3 kg (−0.4 SD) and head circumference was 48.3 cm (−1.1 SD). Brain MRI at 4 years of age showed agenesis of corpus callosum (ACC) (Fig. 3). His DQ was 40. His dysmorphic features including hypertelorism, epicanthal folds, flat nasal bridge, low set ears, growth failure and ACC suggested the diagnosis of Mowat-Wilson syndrome. However, molecular analysis of *ZEB2* mutation was negative. Target gene sequencing revealed a heterozygous mutation in the *PTPN11* gene (c.A188G, p.Y63C). This mutation has been repeatedly reported in Noonan syndrome (NS) (2). We reevaluated his clinical features and concluded that the diagnosis of NS is appropriate. This is the first association of ACC and NS with *PTPN11* mutation.

Other patients

Three patients with cerebellar anomalies were diagnosed with mental retardation and microcephaly with pontine and cerebellar hypoplasia (MICPCH) due to *CASK* mutations. Another patient was homozygous for *AHI1* mutation. The diagnosis of Joubert syndrome was confirmed. They showed typical findings.

Discussion

ACTB mutation in patient 1 was predicted to be pathogenic in *in silico* analysis. BRWS is a rare

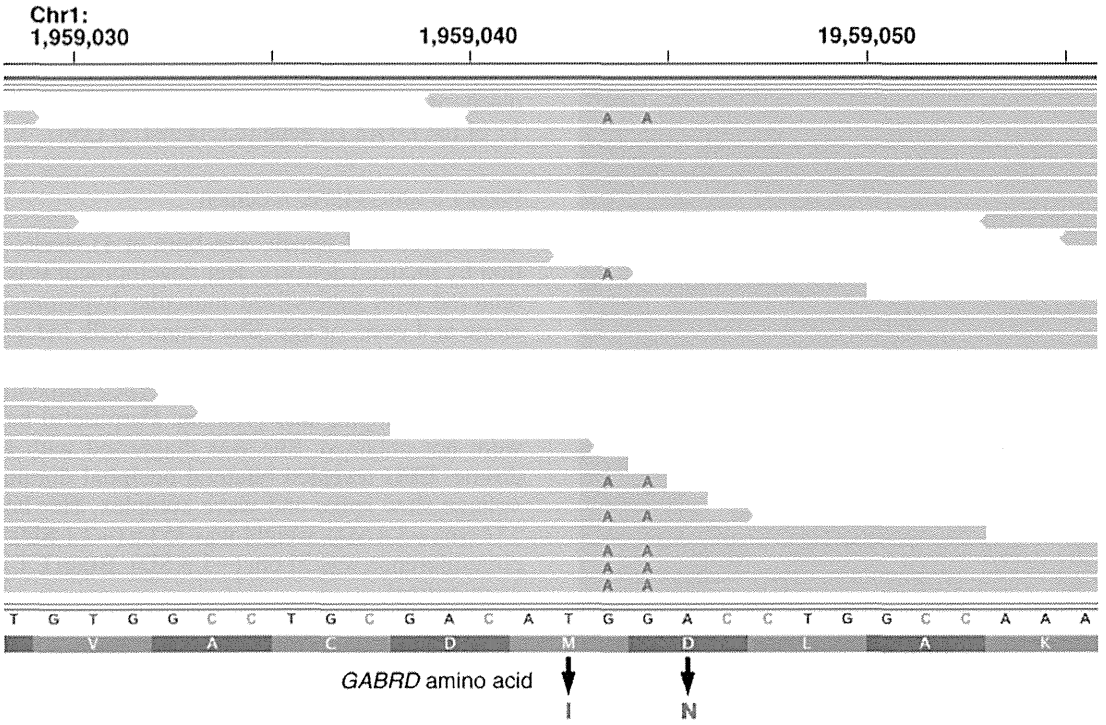

Fig. 2. Patient 3 had 2 bp insertion–deletion corresponding to two amino acids in *GABRD* gene (NM_000815: exon5: c.G498A: p.M166I and NM_000815: exon5: c.G499A: p.D167N).

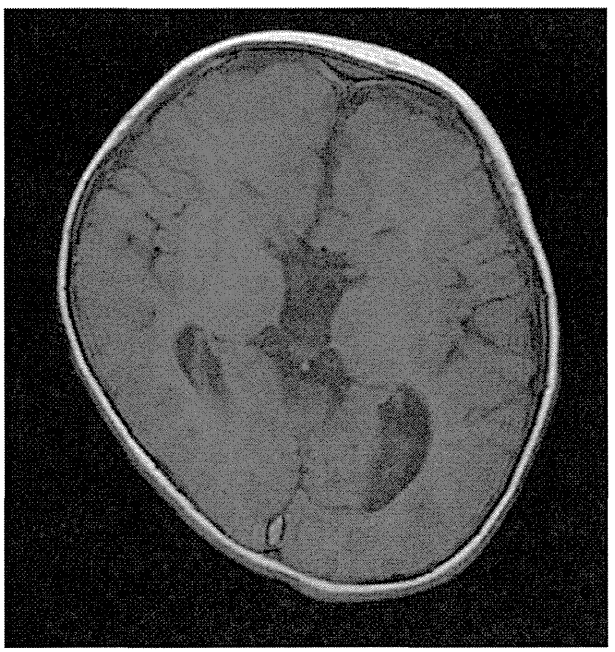

Fig. 3. Brain magnetic resonance imaging (MRI) of patient 4 with *PTPN11* mutation showed agenesis of corpus callosum

MCA/ID syndrome characterized by dysmorphic features, including ptosis, colobomata and neuronal migration anomalies (1). Rivière et al. (3) reported that mutations in *ACTB* and *ACTG1* cause BRWS. Clinical variability of BRWS is often discussed. Di Donato et al. (4) reported three patients with Fryns-Aftimos syndrome (FAS) who had a mutation in the *ACTB* gene. They suggested that mutations in *ACTB* cause a distinctly more severe phenotype than *ACTG1* mutations. They concluded that FAS is an early and severe manifestation of BRWS. Patient 1 did not show the typical features of BRWS. Her dysmorphic features were mild, and her head circumference was over average size. Recently, Verloes et al. (5) delineated the spectrum in 42 patients with BRWS. They reported that facial dysmorphism varies from mild to severe and evolves considerably over times. They suggested the designation of Baraitser-Winter cerebrofrontofacial syndrome.

Patient 2 had had severe ID, motor disturbance, autistic behavior and visual problems. She had truncating mutation of *DYRK1A*. She lacked microcephaly, which was previously assumed to be a constant feature of *DYRK1A* loss of function. *DYRK1A* is a protein kinase that belongs to the highly conserved dual-specificity tyrosine phosphorylation-regulated kinase (DYRK) family. *DYRK1A* is a highly conserved gene located in the Down syndrome critical region at 21q22. *DYRK1A* is involved in brain growth through neuronal proliferation and neurogenesis. *DYRK1A* overexpression has been implicated in ID and microcephaly in Down syndrome.

Haploinsufficiency of *DYRK1A* is associated with ID, epilepsy and microcephaly (6). So far, mutation analysis of *DYRK1A* has been carried out in patients with ID and microcephaly (7, 8). Courset et al. (9) studied the *DYRK1A* gene in a cohort of 105 patients with ID and Angelman syndrome-like symptoms, and they identified a *de novo* frameshift mutation in a patient with growth retardation, ID, and seizures. O'Roak et al. (10)

captured and sequenced 44 candidate genes in 2446 ASD probands. They discovered 27 *de novo* events in 16 genes including *DYRK1A*. The three patients with a *DYRK1A* mutation showed microcephaly.

We suppose that the clinical spectrum of *DYRK1A* mutations may have more variability. Microcephaly may not be a constant feature in the patients with *DYRK1A* mutations. Another novel finding in patient 2 was severe amblyopia. *Dyrk1A* (+/−) mice showed thin retina (11). We recommend ophthalmologic investigation for patients with *DYRK1A* mutations.

Patient 3 had a 2 bp insertion–deletion corresponding to two amino acids in *GABRD* gene. This is the first report of a *GABRD* mutation associated with Rett syndrome like features. *GABRD* encodes a subunit of the ligand-gated chloride channel for gamma-aminobutyric acid (GABA), the major inhibitory neurotransmitter (12). The majority of GABAA receptors contain two α-subunits, two β-subunits, and a γ- or δ-subunit. Mutations in inhibitory GABAA receptor subunit genes (*GABRA1*, *GABRB3*, *GABRG2* and *GABRD*) have been associated with genetic epilepsy syndromes including childhood absence epilepsy (CAE), juvenile myoclonic epilepsy (JME), pure febrile seizures (FS), generalized epilepsy with febrile seizures plus (GEFS+), and Dravet syndrome (or severe myoclonic epilepsy in infancy).

There have been some reports on the association of generalized epilepsies and *GABRD* mutations. *GABRD* gene is assigned to chromosome 1p36 (13). Patients with the 1p36 deletion syndrome often have epileptic seizures (14). Windpassinger et al. (12) found that *GABRD* is expressed most abundantly in the brain. They suggested that the *GABRD* is a good candidate for the neurodevelopmental and neuropsychiatric anomalies seen in the 1p36 deletion syndrome.

Patient 3 has been diagnosed with Rett syndrome. Heterozygous disruption of *GABRB3* produces increased epileptiform EEG activity and elevated seizure susceptibility in Angelman syndrome (15). We assume that mutant *GABRD* is likely to cause increased neuronal excitability in our patient. Further investigation is necessary to clarify mutations in Rett syndrome-like patients without known genetic causes.

Patient 4 was diagnosed with NS, the most common RASopathy characterized by short stature, distinct facial features, congenital heart defect, and ID of various degrees. Patient 4 showed ACC. So far association of NS and ACC is not known. Hypoplasia of corpus callosum is occasionally reported in cardio-facio-cutaneous syndrome, another RASopathy. We consider ACC to be an unusual manifestation of RASopathy.

Our NGS-based mutation screening strategy showed a certain success in the diagnosis of patients with neurodevelopmental disorders when conventional clinical genetic testing has proven negative. Presented patients showed unique or unexpected manifestations. We are planning whole-exome sequencing for the remaining unexplained patients.

Supporting Information

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Acknowledgements

This study was supported by a grant from the Research on Applying Health Technology from the Ministry of Health, Labor and Welfare of Japan. We thank the patients and their families for participating in this work. We thank Mr K. A. Boroevich for English proofreading.

References

1. Baraitser M, Winter RM. Iris coloboma, ptosis, hypertelorism, and mental retardation: a new syndrome. J Med Genet 1988: 25: 41–43.
2. Tartaglia M, Mehler EL, Goldberg R et al. Mutations in PTPN11, encoding the protein tyrosine phosphatase SHP-2, cause Noonan syndrome. Nat Genet 2001: 29: 465–468.
3. Rivière JB, van Bon BW, Hoischen A et al. De novo mutations in the actin genes ACTB and ACTG1 cause Baraitser-Winter syndrome. Nat Genet 2012: 44: 440–444.
4. Di Donato N, Rump A, Koenig R et al. Severe forms of Baraitser-Winter syndrome are caused by ACTB mutations rather than ACTG1 mutations. Eur J Hum Genet 2014: 22: 179–183.
5. Verloes A, Di Donato N, Masliah-Planchon J et al. Baraitser-Winter cerebrofrontofacial syndrome: delineation of the spectrum in 42 cases. Eur J Hum Genet 2014 Jul 23. doi: 10.1038/ejhg.2014.95. [Epub ahead of print]
6. Moller RS, Kubart S, Hoeltzenbein M et al. Truncation of the Down syndrome candidate gene DYRK1A in two unrelated patients with microcephaly. Am J Hum Genet 2008: 82: 1165–1170.
7. Yamamoto T, Shimojima K, Nishizawa T et al. Clinical manifestations of the deletion of Down syndrome critical region including DYRK1A and KCNJ6. Am J Med Genet A 2010: 155A: 113–119.
8. Valetto A, Orsini A, Bertini V et al. Molecular cytogenetic characterization of an interstitial deletion of chromosome 21 (21q22.13q22.3) in a patient with dysmorphic features, intellectual disability and severe generalized epilepsy. Eur J Med Genet 2012: 55: 362–366.
9. Courcet JB, Faivre L, Malzac P et al. The DYRK1A gene is a cause of syndromic intellectual disability with severe microcephaly and epilepsy. J Med Genet 2012: 49: 731–736.
10. O'Roak BJ, Vives L, Fu W, Egertson JD et al. Multiplex targeted sequencing identifies recurrently mutated genes in autism spectrum disorders. Science 2012: 338: 1619–1622.
11. Laguna A, Barallobre MJ, Marchena MÁ et al. Triplication of DYRK1A causes retinal structural and functional alterations in Down syndrome. Hum Mol Genet 2013: 22: 2775–2784.
12. Windpassinger C, Kroisel PM, Wagner K et al. The human gamma-aminobutyric acid A receptor delta (GABRD) gene: molecular characterisation and tissue-specific expression. Gene 2002: 292: 25–31.
13. Emberger W, Windpassinger C, Petek E et al. Assignment of the human GABAA receptor delta-subunit gene (GABRD) to chromosome band 1p36.3 distal to marker NIB1364 by radiation hybrid mapping. Cytogenet Cell Genet 2000: 89: 281–282.
14. Rosenfeld JA, Crolla JA, Tomkins S et al. Refinement of causative genes in monosomy 1p36 through clinical and molecular cytogenetic characterization of small interstitial deletions. Am J Med Genet A 2010: 152A: 1951–1959.
15. DeLorey TM, Olsen RW. GABA and epileptogenesis: comparing gabrb3 gene-deficient mice with Angelman syndrome in man. Epilepsy Res 1999: 36: 123–132.

ELSEVIER

Brain & Development 36 (2014) 159–162

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

www.elsevier.com/locate/braindev

Case report

A case of TUBA1A mutation presenting with lissencephaly and Hirschsprung disease

Norikatsu Hikita [a], Hideji Hattori [b], Mitsuhiro Kato [c], Satoru Sakuma [a], Yoshiki Morotomi [d], Hiroshi Ishida [e], Toshiyuki Seto [a], Katsuji Tanaka [a], Taro Shimono [f], Haruo Shintaku [a], Daisuke Tokuhara [a,*]

[a] *Department of Pediatrics, Osaka City University Graduate School of Medicine, Osaka, Japan*
[b] *Nishinomiya-Sunago Medical and Welfare Center, Hyogo, Japan*
[c] *Department of Pediatrics, Yamagata University Faculty of Medicine, Yamagata, Japan*
[d] *Department of Pediatric Surgery, Osaka City University Graduate School of Medicine, Osaka, Japan*
[e] *Ishida Pediatric Clinic, Osaka, Japan*
[f] *Department of Radiology, Osaka City University Graduate School of Medicine, Osaka, Japan*

Received 28 December 2012; received in revised form 16 February 2013; accepted 19 February 2013

Abstract

Gene mutation of tubulin alpha-1A (TUBA1A), a critical component of microtubules of the cytoskeleton, impairs neural migration and causes lissencephaly (LIS). The approximately 45 cases of disease-associated TUBA1A mutations reported to date demonstrate a wide spectrum of phenotypes. Here we describe an 8-year-old girl with lissencephaly, microcephaly, and early-onset epileptic seizures associated with a novel mutation in the TUBA1A gene. The patient developed Hirschsprung disease and the syndrome of inappropriate antidiuretic hormone secretion (SIADH), which had not previously been described in TUBA1A mutation-associated disease. Our case provides new insight into the wide spectrum of disease phenotypes associated with TUBA1A mutation.

Keywords: TUBA1A; Lissencephaly; Hirschsprung disease; Microcephaly; Syndrome of inappropriate antidiuretic hormone secretion (SIADH)

1. Introduction

Tubulin alpha-1A (TUBA1A) is a critical component of microtubules of the cytoskeleton and is expressed transiently during neuronal development [1]. Because TUBA1A mutation interferes with microtubule function, it is considered to impair neural migration [2]. In 2007, TUBA1A mutations were first proposed to cause human lissencephaly (LIS), which is characterized by a smooth cerebral cortex [2]. The approximately 45 cases of disease-associated TUBA1A mutations reported to date have strengthened the claim that TUBA1A mutations cause LIS in humans [2–5].

The results of several studies have led to the classification of TUBA1A mutation-associated LIS into 2 groups. The first is that of classic LIS, which is characterized by a loosely organized and markedly thickened four-layer cortex and apparently normal cerebellum; the second comprises LIS associated with cerebellar hypoplasia (LCH) [5]. The prevalence of TUBA1A mutations is higher (~30%) in cases of LCH compared with that (1–4%) in cases of classic LIS [3,5]. Recent studies have revealed that, in addition to LIS, TUBA1A mutations can be associated with polymicrogyria,

* Corresponding author. Address: Department of Pediatrics, Osaka City University Graduate School of Medicine, 1-4-3 Asahimachi, Abenoku, Osaka 545-8585, Japan. Tel.: +81 6 6645 3816, fax: +81 6 6636 8737.
E-mail address: m1155519@med.osaka-cu.ac.jp (D. Tokuhara).

0387-7604/$ - see front matter © 2013 The Japanese Society of Child Neurology. Published by Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.braindev.2013.02.006

epilepsy, developmental delay, facial dysmorphy, facial diplegia, and other abnormalities, demonstrating a wide spectrum of clinical phenotypes [4,6]. However, because of the limited number of cases, it remains necessary to accumulate cases of disease-associated TUBA1A mutation and clarify the mutation-specific spectrum of the clinical phenotype. In addition, several polymorphisms of TUBA1A are unrelated to disease [3]. To establish appropriate genetic counseling, information regarding disease-associated TUBA1A mutations should be evaluated through detailed case studies [3].

Here we present a case that is the first known to manifest LIS and microcephaly in combination with the syndrome of inappropriate antidiuretic hormone secretion (SIADH), Hirschsprung disease (HSCR), and a novel mutation in the TUBA1A gene. We provide new insight into the wide spectrum of clinical phenotypes of TUBA1A mutations.

2. Case report

The patient is an 8-year-old girl who was born to healthy nonconsanguineous parents by vaginal delivery at a gestational age of 42 weeks with Apgar scores of 7 at 1 min and 8 at 5 min. She has no siblings. Her birth weight was 2.176 g (−3.1 SD), crown–heel length was 48.5 cm (−0.6 SD), and occipitofrontal head circumference was 29 cm (−3.5 SD), thus showing symmetrical intrauterine growth retardation. Facial dysmorphism included microcephaly, hypertelorism, and large uplifted earlobes. On day 9 after birth, she developed infantile seizures, which were controlled by using two antiepileptic drugs. Magnetic resonance imaging (MRI) of the brain disclosed agyria, enlarged lateral ventricles, agenesis of the corpus callosum, abnormal rotation of the hippocampus, dysmorphic and hypoplastic features of the basal ganglia and thalamus, and hypoplastic cerebellum which led to a diagnosis of LCH (Fig. 1).

When the patient was 6 months old, seizures recurred and were found to be associated with hyponatremia (122 mEq/L), increased body weight, high urinary sodium (140 mEq/L; normal, <20 mEq/L), low serum osmolarity (252 mOsm; normal, 260–290 mOsm), and high urine osmolarity (360 mOsm) relative to the serum osmolarity—features consistent with the diagnostic criteria for SIADH. Fluid restriction treatment successfully resolved the hyponatremia, and the seizures diminished simultaneously. However, she concurrently developed abdominal distention and increasing vomiting. A barium enema showed a narrowed segment of the rectum (Fig. 2). A rectal biopsy demonstrated an absence of ganglion cells and an increase in the number and size of acetylcholinesterase-positive nerve fibers in the lamina propria. In light of the findings, HSCR was diagnosed, and supportive care with enemas and central vein catheterization were initiated, without surgical intervention. At the age of 8 years, she is undergoing treatment with tube feeding and anti-epileptic drugs. She shows severely retarded mental and physical development with poor social interaction. Chromosome analysis showed a normal 46, XX karyotype in all cells. Testing for LIS1 and ZFHX1B2 gene mutations (for LIS and Mowat–Wilson syndrome, respectively) produced negative results. Evaluation of TUBA1A revealed a heterozygous c.599G→A (p.Cys402Tyr) mutation. Parental samples were not obtained, however the heterozygous c.599G→A (p.Cys402Tyr) mutation was absent in 22 healthy control individuals.

3. Discussion

TUBA1A mutation-associated LIS is a rare genetic disorder. We have added a new case with a confirmed novel TUBA1A mutation, which was not identified in 22 healthy control individuals in our study and 60 healthy control individuals in a previous study [3]. Brain MRI of the present case showed complete or near-complete agyria, corresponding to classic LIS grade 1 associated with cerebellar hypoplasia; together, these can be categorized as LCH. LCH is predominantly associated with mutation of TUBA1A rather than LIS1 and DCX [5]; therefore the brain MRI findings in the present case are consistent with the predominant TUBA1A mutation-associated phenotype. According to previous studies, the site of the mutation in TUBA1A seems to determine the brain disorder phenotype [4,5]. For example, all 5 cases associated with the TUBA1A mutation p.R402C showed LIS, whereas all 4 cases associated with the mutation p.R402H manifested LCH [5]. In another study, 2 cases with the mutation p.R264C showed pachygyria with subcortical band heterotopia, vermis hypoplasia of the cerebellum, and partial agenesis of the corpus callosum [4]. Accordingly, our case, which is associated with the novel mutation p.C402Y, will facilitate prediction of the brain disorder phenotype in a future case with the same mutation.

The most striking feature of the case is the association of LIS with HSCR. Only one other case presenting with both LIS and HSCR has been reported; that case also showed microcephaly and tetralogy of Fallot, although the underlying gene mutation was unknown [7]. Among cases of LIS due to genetic mutation (in LIS1, DCX and so on), ours is the first to manifest both LIS and HSCR, thus the present case implicates that HSCR can be included among the clinical phenotypes of TUBA1A mutation-associated disease. HSCR, which is characterized by a lack of ganglion cells in the bowel, is a multigenic neurocristopathy due to failure of neural crest cell migration [8]. Although TUBA1A mutation is well known to

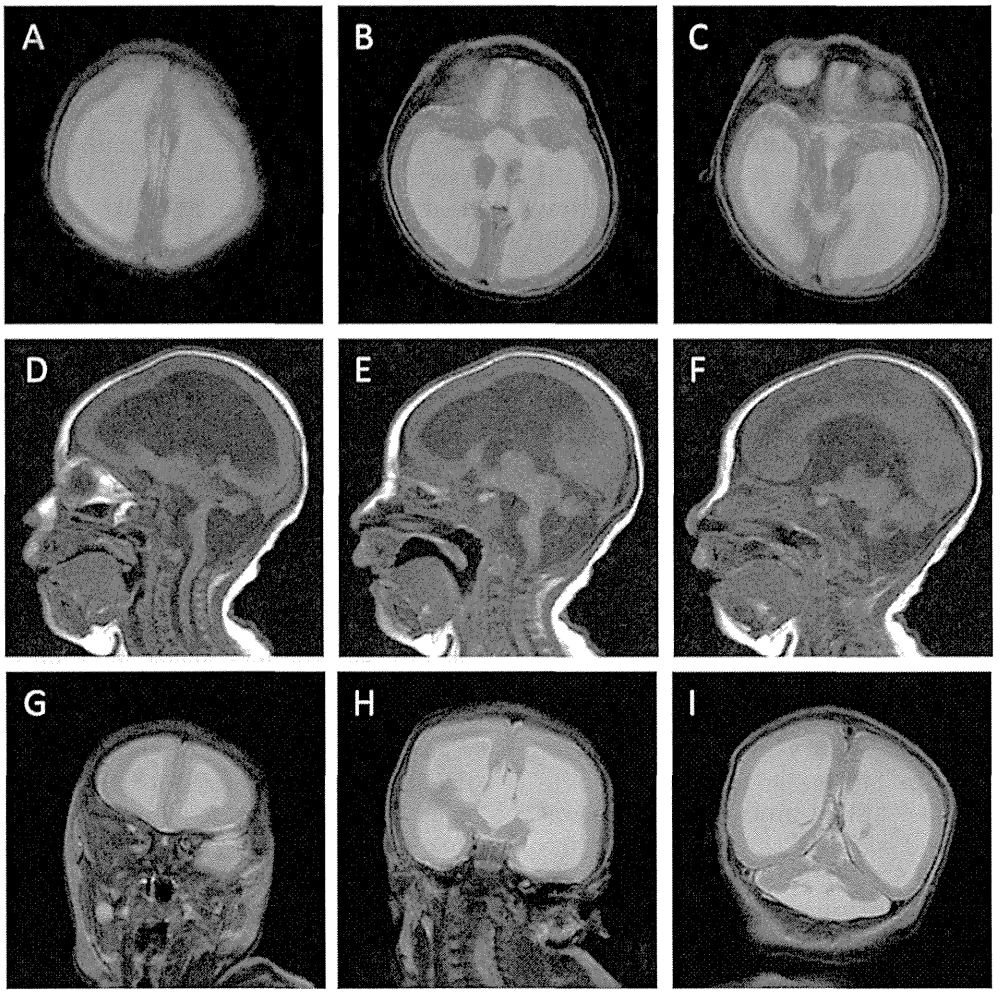

Fig. 1. T2 weighted Axial (A, B and C), T1 weighted sagital (D, E and F) and T2 weighted coronal (G, H and I) images by MRI showing agyria (grade I) (A-I), agenesis of the corpus callosum (D-F, H), excessive dilation of lateral ventricles (A, B, C, D, G, H, I), abnormal rotation of hippocampus (H), the basal ganglia and thalamus of the dysmorphic and hypoplastic features (B, H), cerebral aqueduct stenosis (E), cerebellum posterior rotation, and pontocerebellar hypoplasia (D, E, F, I).

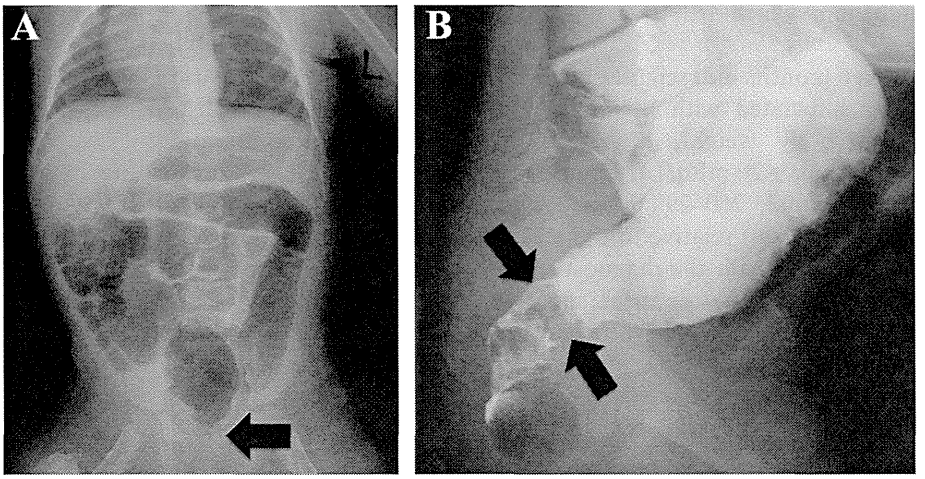

Fig. 2. (A) Frontal abdominal radiograph showing marked dilation of the bowel with absence of gas in the rectum (arrow). (B) Lateral view of a barium enema examination at 6 months of age disclosed abnormal narrowing of the rectum (arrow).

impair neural migration and subsequently cause LIS, no study to date has clarified the correlation between TUBA1A mutation and abnormal neural crest cell migration in leading to HSCR. However, a recent study demonstrated that the gap junction protein connexin 43 (CX43) affects neural crest migration via effects on microtubules [9]. The CX43 protein is expressed abundantly in migrating neural crest cells, which are functionally coupled through gap junction channels [10]. CX43-knockout mice showed cardiac anomalies that arose from the abnormal deployment of cardiac neural crest [11]. Another study demonstrated the loss of CX43 in the aganglionic intestinal segments in HSCR [12]. In addition, microtubules have been identified as playing a critical role in the trafficking of CX43 to the cell membrane [13]. Furthermore, CX43 binds to both alpha-tubulin and beta-tubulin [14]. Together, these findings suggest that mutation of TUBA1A, which is a critical component of microtubules, alters neural crest migration through impairment of CX43 function and thus contributes to the development of HSCR.

Our patient had SIADH, which occurs in patients with diverse medical conditions, including agenesis of the corpus callosum, bronchial carcinoma, hydrocephalus, and postoperative brain injury among others [15]. Because SIADH is a secondary disease rather than a specific genetic disorder, we speculate that our patient's SIADH arose due to her severe brain malformations and was not a direct result of TUBA1A mutation.

In conclusion, we present a patient with a novel TUBA1A mutation associated with LIS and HSCR. Our case provides new insight into the wide spectrum of disease phenotypes and implicates TUBA1A in both neuronal and neural crest migration. TUBA1A gene analysis should be considered in cases of LIS associated with HSCR in the absence of known gene mutations.

Acknowledgement

We thank Dr. Naoya Morisada (Department of Pediatrics, Kobe University Graduate School of Medicine) for ZFHX1B2 gene analysis.

References

[1] Coksaygan T, Magnus T, Cai J, Mughal M, Lepore A, Xue H, et al. Neurogenesis in Tα-1 tubulin transgenic mice during development and after injury. Exp Neurol 2006;197:475–85.
[2] Keays DA, Tian G, Poirier K, Huang GJ, Siebold C, Cleak J, et al. Mutations in α-tubulin cause abnormal neuronal migration in mice and lissencephaly in humans. Cell 2007;128:45–57.
[3] Morris-Rosendahl DJ, Najm J, Lachmeijer AMA, Sztriha L, Martins M, Kuechler A, et al. Refining the phenotype of α-1a Tubulin (TUBA1A) mutation in patients with classical lissencephaly. Clin Genet 2008;74:425–33.
[4] Sohal APS, Montgomery T, Mitra D, Ramesh V. TUBA1A mutation-associated lissencephaly: case report and review of the literature. Pediatr Neurol 2012;46:127–31.
[5] Kumar RA, Pilz DT, Babatz TD, Cushion TD, Harvey K, Topf M, et al. TUBA1A mutations cause wide spectrum lissencephaly (smooth brain) and suggest that multiple neuronal migration pathways converge on alpha tubulins. Hum Mol Genet 2010;19:2817–27.
[6] Poirier K, Saillour Y, Fourniol F, Francis F, Souville I, Valence S, et al. Expanding the spectrum of TUBA1A-related cortical dysgenesis to polymicrogyria. Eur J Hum Genet, in press. http://dx.doi.org/10.1038/ejhg.2012.195.
[7] Tanteles GA, Kurup B, Rauch A, Splitt MP. Microcephaly, lissencephaly, Hirschsprung disease, and tetralogy of Fallot: a new syndrome? Clin Dysmorphol 2006;15:107–10.
[8] Amiel J, Lyonnet S. Hirschsprung disease, associated syndromes, and genetics: a review. J Med Genet 2001;38:729–39.
[9] Francis R, Xu X, Park H, Wei CJ, Chang S, Chatterjee B, et al. Connexin43 modulates cell polarity and directional cell migration by regulating microtubule dynamics. PLoS One 2011;6:e26379.
[10] Lo CW, Waldo KL, Kirby ML. Gap junction communication and the modulation of cardiac neural crest cells. Trends Cardiovasc Med 1999;9:63–9.
[11] Clauss SB, Walker DL, Kirby ML, Schimel D, Lo CW. Patterning of coronary arteries in wildtype and connexin43 knockout mice. Dev Dyn 2006;235:2786–94.
[12] Nemeth L, Maddur S, Puri P. Immunolocalization of the gap junction protein connexin 43 in the interstitial cells of cajal in the normal and Hirschsprung's disease bowel. J Pediatr Surg 2000;35:823–8.
[13] Thomas T, Jordan K, Simek J, Shao Q, Jedeszko C, Walton P, et al. Mechanisms of Cx43 and Cx26 transport to the plasma membrane and gap junction regeneration. J Cell Sci 2005;118:4451–62.
[14] Giepmans BNG, Verlaan I, Moolenaar WH. Connexin-43 interactions with ZO-1 and α- and β-tubulin. Cell Commun Adhes 2001;8:219–23.
[15] Amini A, Schmidt MH. Syndrome of inappropriate secretion of antidiuretic hormone and hyponatremia after spinal surgery. Neurosurg Focus 2004;16:E10.