Ⅲ．研究成果の刊行に関する一覧表

研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
加藤光広	脳・脊髄形成異常、皮質形成異常、Dandy-Walker奇形、Chiari奇形、二分脊椎	遠藤文夫	小児科診断・治療指針	中山書店	東京	2012	744-748
加藤光広	小脳奇形		小児疾患の診断治療基準第4版	東京医学社	東京	2012	678-679
白石秀明	急性散在性脳脊髄炎、多発性硬化症	遠藤文夫	小児科　診断・治療指針	中山書店	東京	2012	741-744
遠山　潤,柿田明美	動脈管開存の既往があり，難治性てんかんを示した女性例		小児神経学の進歩第41集	診断と治療社	東京	2012	78-101
鳥巣浩幸、原寿郎	小児多発性硬化症.	辻省次	アクチュアル脳・神経疾患の臨床最新アプローチ多発性硬化症と視神経脊髄炎	中山書店	東京	2012	85-91

雑誌

発表者氏名	論文タイトル名	発表誌名	巻号	ページ	出版年
Yoneda Y, et al.	Phenotypic spectrum of COL4A1 mutations: porencephaly to schizencephaly	*Ann Neurol*	73	48-57	2013
Saitsu H, Kato M, et al.	Whole exome sequencing identifies *KCNQ2* mutations in Ohtahara syndrome.	*Ann Neurol*	72	298-300	2012
Saitsu H, Kato M, et al.	*CASK* aberrations in male patients with Ohtahara syndrome and cerebellar hypoplasia.	*Epilepsia*	53	1441-1449	2012
Yoneda Y, Haginoya K, et al.	De Novo and Inherited Mutations in *COL4A2*, Encoding the Type IV Collagen alpha2 Chain Cause Porencephaly.	*Am J Hum Genet*	90	86-90	2012
Saitsu H*, Kato M*, et al. (*contributed equally)	Association of genomic deletions in the *STXBP1* gene with Ohtahara syndrome.	*Clin Genet*	81	399-402	2012
Tsurusaki Y, Okamoto N, et al.	Mutations affecting components of the SWI/SNF complex cause Coffin-Siris syndrome.	*Nat Genet*	44	376-378	2012
Nakamura K, Kato M, et al.	Congenital dysplastic microcephaly and hypoplasia of the brainstem and cerebellum with diffuse intracranial calcification.	*J Child Neurol*	27	218-221	2012

Honda S, Hayashi S, et al.	The incidence of hypoplasia of the corpus callosum in patients with dup(X)(q28) involving *MECP2* is associated with the location of distal breakpoints.	*Am J Med Genet A*	158A	1292-1303	2012
Shiraishi H, Haginoya K, et al.	Magnetoencephalography localizing spike sources of atypical benign partial epilepsy.	Brain Dev	in press		
Takahashi Y, Sugiyama M, et al.	Childhood-onset anti-MuSK antibody positive myasthenia gravis demonstrates a distinct clinical course.	Brain Dev	34	784-786	2012
Sueda K, Takeuchi F, Shiraishi H, et al.	Magnetoencephalographic analysis of paroxysmal fast activity in patients with epileptic spasms.	Epi Res	104	68-77	2013
Ozaki I, Shiraishi H, et al.	Publication criteria for evoked magnetic fields of the human brain. A proposal.	Clin Neurophysiol	123	2116-2121	2012
Higurashi N, Shi X, et al.	*PCDH19* mutation in Japanese females with epilepsy.	Epi Res	99	28-37	2012
Asano T, Kuroda S, et al.	[A case of vein of Galen aneurysmal malformation with a newly developed dural arteriovenous fistula after successful embolization].	No Shinkei Geka.	40	519-525	2012
Tsurusaki Y, Saitoh S, et al.	A *DYNC1H1* mutation causes a dominant spinal muscular atrophy with lower extremity.	Neurogenetics.	13	327-332	2012
Okumura A, Uematsu M, et al.	Acute encephalopathy in children with Drave syndrome.	Epilepsia	53	79-86	2012
Masuda H, Shariff E, et al.	Clinical patterns and pathophysiology of hypermotor seizures. An ictal SPECT study.	Epileptic Disord	14	32-40	2012
Kobayashi Y, Ohashi T, et al.	Congenital variant of Rett syndrome due to an intragenic large deletion in MECP2.	Brain Dev	34	601-604	2012
Ogura K, Fujii T, et al.	Regional cerebral blood flow and abnormal eating behavior in Prader-Willi syndrome.	Brain Dev	in press		
Takanashi J, Okamoto N, et al.	Clinical and radiological features of Japanese patients with a severe phenotype due to CASK mutations.	*Am J Med Genet A*	158A	3112-3118	2012
Shimojima K, Mano T, et al.	Pelizaeus-Merzbacher disease caused by a duplication-inverted triplication-duplication in chromosomal segments including the PLP1 region.	*Eur J Med Genet*	55	400-403	2012
Torisu H, Iwaki A, et al.	Clinical and genetic characterization of a 2-year-old boy with complete PLP1 deletion.	Brain Dev	34	852-856	2012
Torisu H, Yoshikawa Y, et al.	Alexander disease with mild dorsal brainstem atrophy and infantile spasms.	Brain Dev	in press		

Sanefuji M, Torisu H, et al.	A case of childhood stiff-person syndrome with striatal lesions. A possible entity distinct from the classical adult form.	Brain Dev	in press		
Ishizaki Y, Yukaya N, et al.	PD1 as a common candidate susceptibility gene of subacute sclerosing panencephalitis.	Hum Genet	in press		
Iwami T, Kitamura T, et al.	Chest compression-only cardiopulmonary resuscitation for out-of-hospital cardiac arrest with public-access defibrillation. A nationwide cohort study.	Circulation	in press		
Kitamura T, Iwami T, et al.	Nationwide improvements in survival form out-of-hospital cardiac arrest in Japan.	Circulation	in press		
Yoshimizu M, Teo A, et al.	Relief of chronic shoulder and neck pain by electro-acupuncture and transcutaneous electrical nervous stimulation. A randomized crossover trial.	Medical Acupuncture	24	97-103	2012
Wakai K, Naito M, et al.	Tooth loss and risk of hip fracture. A prospective study of male Japanese dentists.	Community Dent Oral Epidemiol	in press		
Kiyohara K, Kawamura T, et al.	Changes in teachers' smoking behaviour following enforcement of a total smoke-free school policy.	Public Health	126	678-681	2012
加藤光広	乳幼児期てんかん性脳症の分子遺伝学的発症機構	北海道小児神経症例検討会10周年記念誌		189-191	2012
加藤光広	ARX遺伝子と介在ニューロン病.抑制性シナプスの基礎と臨床	Clinical Neuroscience	30(12)	1401-1403	2012
露口尚弘，他	脳磁図の臨床応用に関する文献レビュー（第２報）虚血性脳血管障害	臨床神経生理	40	195-202	2012
白石秀明，他	脳磁図の臨床応用に関する文献レビュー（第３報）小児疾患	臨床神経生理	40	203-208	2012
白石秀明，他	本邦における脳磁図検査施行の実態とその問題点	臨床神経生理	40	119-130	2012
白石秀明	熱性けいれんはてんかんなのでしょうか？	治療	94	1737-1738	2012
小林悠，遠山潤	てんかん	こどもケア	6	14-22	2012
遠山潤	朝に出現する全身性けいれん	小児科診療	75	1419-1423	2012
小倉加恵子	発達障害概説およびリハビリテーションが関わりうる課題	リハビリテーション医学	49	895-898	2012
荒井洋	乳幼児健診Q&A　成長発達	小児科診療	75	1839-1842	2012
鳥巣浩幸	小児の診察法　正しい大泉門の診察法を教えてください.	小児内科	43	97-99	2012

Ⅳ. 研究成果の刊行物・別刷

Phenotypic Spectrum of *COL4A1* Mutations: Porencephaly to Schizencephaly

Yuriko Yoneda, MSc,[1] Kazuhiro Haginoya, MD, PhD,[2,3] Mitsuhiro Kato, MD, PhD,[4] Hitoshi Osaka, MD, PhD,[5] Kenji Yokochi, MD, PhD,[6] Hiroshi Arai, MD,[7] Akiyoshi Kakita, MD, PhD,[8] Takamichi Yamamoto, MD, MS, DMSc,[9] Yoshiro Otsuki, MD, PhD,[10] Shin-ichi Shimizu, DDS, PhD,[10] Takahito Wada, MD, PhD,[5] Norihisa Koyama, MD, PhD,[11] Yoichi Mino, MD,[12] Noriko Kondo, MD,[13] Satoru Takahashi, MD, PhD,[14] Shinichi Hirabayashi, MD, PhD,[15] Jun-ichi Takanashi, MD, PhD,[16] Akihisa Okumura, MD, PhD,[17] Toshiyuki Kumagai, MD,[18] Satori Hirai, MD,[7] Makoto Nabetani, MD,[19] Shinji Saitoh, MD, PhD,[20] Ayako Hattori, MD, PhD,[20] Mami Yamasaki, MD, PhD,[21] Akira Kumakura, MD,[22] Yoshinobu Sugo, MD,[1] Kiyomi Nishiyama, PhD,[1] Satoko Miyatake, MD, PhD,[1] Yoshinori Tsurusaki, PhD,[1] Hiroshi Doi, MD, PhD,[1] Noriko Miyake, MD, PhD,[1] Naomichi Matsumoto, MD, PhD,[1] and Hirotomo Saitsu, MD, PhD[1]

Objective: Recently, *COL4A1* mutations have been reported in porencephaly and other cerebral vascular diseases, often associated with ocular, renal, and muscular features. In this study, we aimed to clarify the phenotypic spectrum and incidence of *COL4A1* mutations.
Methods: We screened for *COL4A1* mutations in 61 patients with porencephaly and 10 patients with schizencephaly, which may be similarly caused by disturbed vascular supply leading to cerebral degeneration, but can be distinguished depending on time of insult.
Results: *COL4A1* mutations were identified in 15 patients (21%, 10 mutations in porencephaly and 5 mutations in schizencephaly), who showed a variety of associated findings, including intracranial calcification, focal cortical dysplasia, pontocerebellar atrophy, ocular abnormalities, myopathy, elevated serum creatine kinase levels, and hemolytic anemia. Mutations include 10 missense, a nonsense, a frameshift, and 3 splice site mutations. Five mutations were confirmed as de novo events. One mutation was cosegregated with familial porencephaly, and 2

View this article online at wileyonlinelibrary.com. DOI: 10.1002/ana.23736

Received Jun 8, 2012, and in revised form Aug 6, 2012. Accepted for publication Aug 10, 2012.

Address correspondence to Dr Saitsu, Department of Human Genetics, Yokohama City University Graduate School of Medicine, 3-9 Fukuura, Kanazawa-ku, Yokohama 236-0004, Japan. E-mail: hsaitsu@yokohama-cu.ac.jp

From the [1]Department of Human Genetics, Yokohama City University Graduate School of Medicine, Yokohama; [2]Department of Pediatrics, Tohoku University School of Medicine, Sendai; [3]Department of Pediatric Neurology, Takuto Rehabilitation Center for Children, Sendai; [4]Department of Pediatrics, Yamagata University Faculty of Medicine, Yamagata; [5]Division of Neurology, Clinical Research Institute, Kanagawa Children's Medical Center, Yokohama; [6]Department of Pediatric Neurology, Seirei-Mikatahara General Hospital, Hamamatsu; [7]Department of Pediatric Neurology, Morinomiya Hospital, Osaka; [8]Department of Pathology, Brain Research Institute, University of Niigata, Niigata; [9]Comprehensive Epilepsy Center; [10]Department of Pathology, Seirei Hamamatsu General Hospital, Shizuoka; [11]Department of Pediatrics, Toyohashi Municipal Hospital, Toyohashi; [12]Division of Child Neurology, Tottori University Faculty of Medicine, Yonago; [13]Division of Child Neurology, Department of Brain and Neurosciences, Tottori University Faculty of Medicine, Yonago; [14]Department of Pediatrics, Asahikawa Medical University, Asahikawa; [15]Department of Neurology, Nagano Children's Hospital, Azumino; [16]Department of Pediatrics, Kameda Medical Center, Chiba; [17]Department of Pediatrics, Juntendo University Faculty of Medicine, Tokyo; [18]Aichi Welfare Center for Persons with Developmental Disabilities, Kasugai; [19]Department of Pediatrics, Yodogawa Christian Hospital, Osaka; [20]Department of Pediatrics and Neonatology, Nagoya City University Graduate School of Medical Sciences, Nagoya; [21]Department of Pediatric Neurosurgery, Takatsuki General Hospital, Osaka; and [22]Department of Pediatrics, Kitano Hospital, Tazuke Kofukai Medical Research Institute, Osaka, Japan.

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

mutations were inherited from asymptomatic parents. Aberrant splicing was demonstrated by reverse transcriptase polymerase chain reaction analyses in 2 patients with splice site mutations.
Interpretation: Our study first confirmed that *COL4A1* mutations are associated with schizencephaly and hemolytic anemia. Based on the finding that *COL4A1* mutations were frequent in patients with porencephaly and schizencephaly, genetic testing for *COL4A1* should be considered for children with these conditions.

ANN NEUROL 2013;73:48–57

Type IV collagens are basement membrane proteins that are expressed in all tissues, including the vasculature. COL4A1 (α1 chain) and COL4A2 (α2 chain) are the most abundant type IV collagens, and form heterotrimers with a 2:1 stoichiometry (α1α1α2).[1] Mutations in *COL4A1* and *COL4A2* cause sporadic and hereditary porencephaly, a neurological disorder characterized by fluid-filled cysts in the brain that often cause hemiplegia or tetraplegia.[2–4] In addition, a variety of clinical phenotypes, including small vessel disease affecting the brain, eyes, and kidneys, are associated with *COL4A1* abnormality[5,6]: neonatal porencephaly and adult stroke,[7] sporadic extensive bilateral porencephaly resembling hydranencephaly,[8] periventricular leukomalacia with intracranial calcification,[9] HANAC (hereditary angiopathy with nephropathy, aneurysm, and muscle cramps) syndrome,[10,11] Axenfeld–Rieger anomaly with leukoencephalopathy, and adult stroke and intracerebral hemorrhage.[12–14] Notably, *COL4A1* mutations were present in 2 patients with muscle–eye–brain/Walker–Warburg syndrome (MEB/WWS), which is characterized by ocular dysgenesis, neuronal migration defects, and congenital muscular dystrophy, suggesting that *COL4A1* is also involved in normal cortical and muscular development in humans.[15] Consistent with this hypothesis, a mouse model of a heterozygous *COL4A1* mutation (*Col4a1*$^{+/\Delta ex40}$) showed ocular dysgenesis, cortical neuronal localization defects, and myopathy, along with cerebral hemorrhage and porencephaly.[2,15] The phenotypic spectrum of *COL4A1* mutations is expanding; however, the whole spectrum of systemic phenotypes and the incidence of *COL4A1* mutations associated with porencephaly has not been systemically examined.

In this study, we screened for *COL4A1* mutations in 61 patients with porencephaly and 10 patients with schizencephaly, which may be similarly caused by disturbed vascular supply leading to cerebral degeneration, but can be distinguished depending on time of insult.[2–4,16,17] *COL4A1* mutations were identified in 10 patients with porencephaly and 5 patients with schizencephaly, who showed a variety of associated findings, including intracranial calcification, focal cortical dysplasia (FCD), ocular abnormalities, pontocerebellar atrophy, myopathy, elevated serum creatine kinase levels, and hemolytic anemia. Our study demonstrated the importance of genetic testing for *COL4A1* in children with porencephaly or schizencephaly.

Patients and Methods

Patients

A total of 61 patients with porencephaly including a previous cohort with porencephaly,[4] and 10 patients with schizencephaly including a patient who also had porencephaly were analyzed for *COL4A1* mutations. Schizencephaly is defined as transmantle clefts bordered by polymicrogyria in adjacent cortex.[18] The clefts extended through the entire hemisphere, from the ependymal lining of the lateral ventricles to the pial covering of the cortex.[19] The clefts are further divided into those with closed lips and those with open lips. In the clefts with closed lips, the walls affix each other directly, obliterating the cerebrospinal fluid space within the cleft at that point.[20] *COL4A2* mutations were negative for these patients. Genomic DNA was isolated from blood leukocytes according to standard methods, and amplified using an illustra GenomiPhi V2 DNA Amplification Kit (GE Healthcare, Buckinghamshire, UK). The DNA of familial members of patient 6 was isolated from saliva samples using Oragene (DNA Genotek, Kanata, Ontario, Canada). Experimental protocols were approved by the committee for ethical issues at Yokohama City University School of Medicine. All patients were investigated in agreement with the requirements of Japanese regulations.

Mutation Analysis

Exons 1 to 52, covering the entire *COL4A1* coding region, were examined by high-resolution melting (HRM) curve analysis. Samples showing an aberrant melting curve pattern in the HRM analysis were sequenced. Polymerase chain reaction (PCR) primers and conditions are shown in Supplementary Table S1. All novel mutations were verified using original genomic DNA, and screened in 200 Japanese normal controls by HRM analysis. For the family showing de novo mutations, parentage was confirmed by microsatellite analysis, as previously described.[21] Biological parents were confirmed if >4 informative markers were compatible and other markers showed no discrepancy.

Reverse Transcriptase-PCR

Reverse transcriptase (RT)-PCR using total RNA extracted from lymphoblastoid cell lines (LCL) was performed essentially as previously described.[22] Briefly, total RNA was extracted using RNeasy Plus MiniKit (Qiagen, Tokyo, Japan) from LCL with or without 30μM cycloheximide (CHX; Sigma, Tokyo, Japan) incubation for 4 hours. Four micrograms total RNA was subjected to reverse transcription, and 2μl cDNA was used for PCR. Primer sequences are ex20-F (5′-CCCAAAAGGTTTCC CAGGACTACCA-3′) and ex22-R (5′-GTCCGGGCTGACAT TCCACAATTC-3′; for patient 4); and ex22-F (5′-GAATTC-CAGGGCAGCCAGGATTTAT-3′) and ex24-R (5′-CATCTCT GCCAGGCAAACCTCTGT-3′; for patient 7). DNA of each

PCR band was purified by QIAEXII Gel extraction kit (Qiagen; for patient 4) and E.Z.N.A. poly-Gel DNA Extraction kit (Omega Bio-Tek, Norcross, GA; for patient 7), respectively.

Results

Mutation and RT-PCR analysis

COL4A1 abnormalities were identified in 15 patients (Fig 1 and Table). Nine mutations occurred at highly conserved Gly residues in the Gly-X-Y repeat of the collagen triple helical domain. Interestingly, a missense mutation (c.4843G>A [p.Glu1615Lys]) at an evolutionary conserved amino acid and a nonsense mutation (c.4887C>A [p.Tyr1629X]) were found in the carboxy-terminal noncollagenous (NC1) domain. The other 4 mutations include a frameshift mutation (c.2931dupT [p.Gly978TrpfsX15]) and 3 splice site mutations (c.1121-2dupA, c.1382-1G>C, and c.1990+1G>A). None of these mutations was present in 200 Japanese normal controls, and Web-based prediction tools suggested that these mutations are pathogenic (Supplementary Table S2). The c.2842G>A (patient 1), c.3976G>A (patient 2), c.4887C>A (patient 8), c.2689G>A (patient 13), and c.1990+1G>A (patient 14) mutations occurred de novo. The c.3995G>A mutation (patient 3) was not found in the mother's DNA (the father's DNA was unavailable). The c.1121-2dupA (patient 4) and c.2931dupT (patient 6) mutations were found in the asymptomatic fathers. c.1963G>A (patient 10) was found in familial members affected with porencephaly as well as asymptomatic carriers, suggesting incomplete penetrance of the mutation (Supplementary Fig S1). The remaining patients' parental DNA was unavailable.

To examine the mutational effects of the 2 splice acceptor site mutations (c.1121-2dupA and c.1382-1G>C), RT-PCR and sequencing were performed (see Fig 1). c.1121-2dupA caused the deletion of exon 21 from the wild-type *COL4A1* mRNA, resulting in an in-frame 55-amino acid deletion (p.Gly374_Asn429delinsAsp). The effect of c.1382-1G>C was more complicated. There were 3 PCR products amplified from LCL treated with CHX, which inhibits nonsense-mediated mRNA decay (NMD). The middle band corresponded to the wild-type allele. The sequence of the lower mutant band showed a 33bp insertion of intron 22 and an 84bp deletion of all of exon 23 from the use of cryptic splice acceptor and donor sites within intron 22. The change of amino acid sequence from this mutant transcript was a deletion of 29 amino acids and an insertion of 12 amino acids (p.Gly461_Gly489delinsValHisCysGlyAspPheTrpSerHisValThrArg). The upper band was only observed in CHX-treated LCL, but was not evident in the untreated LCL, suggesting that this mutant transcript may undergo NMD. Sequencing of the upper band showed a 61bp insertion of intron 22 from the use of a cryptic splice acceptor site within intron 22, as mentioned above. The product of this mutant transcript leads to a frameshift, creating a premature stop codon (p.Gly461ValfsX31), which is consistent with degradation of the mutant transcript by NMD.

Clinical Features

The clinical information for individuals with *COL4A1* mutations is summarized in the Table, and their representative brain images are shown in Figure 2 and Supplementary Fig S2. *COL4A1* mutations were identified in 10 of 61 patients with porencephaly (16.4%). Of note, *COL4A1* mutations were identified in 5 of 10 patients with schizencephaly (50.0%), revealing a novel association between *COL4A1* mutations and schizencephaly. Thirteen patients were born at term, and 2 patients (patients 1 and 12) were born at preterm. Their body weight was normal at birth except for 5 patients (patients 3, 4, 9, 12, and 15) who were below −2.0 standard deviations. The occipitofrontal circumference was available in 12 patients, and 6 patients (patients 2, 3, 6, 13, 14, and 15) were below −2.0 standard deviations. Two patients (patients 11 and 12) were confirmed to have an antenatal hemorrhage as previously reported.[23,24] Among associated findings with *COL4A1* mutations, a patient showed FCD that was histologically demonstrated (Fig 3A–F). In addition, hemolytic anemia was found in 5 of 15 patients, suggesting that hemolytic anemia may be a novel feature associated with *COL4A1* mutation. Pontocerebellar atrophy along with severe bilateral porencephaly was observed in 2 patients, and a patient showed cerebellar hypoplasia. Previously reported magnetic resonance imaging and systemic findings associated with *COL4A1* mutations were also observed, including intracranial calcification (7 of 15), myopathy (1 of 15; see Fig 3G, H), ocular abnormalities (4 of 15), and elevated serum creatine kinase levels (6 of 15), confirming that these features are useful signs for *COL4A1* testing. Case reports are available in the Supplementary Data.

Discussion

We found a total of 15 novel mutations in this study. Nine mutations occurred at highly conserved Gly residues in the Gly-X-Y repeat of the collagen triple helical domain, suggesting that these mutations may alter the collagen IV $\alpha1\alpha1\alpha2$ heterotrimers.[1,25] We reported for the first time 2 mutations (a nonsense and a missense change) in the NC1 domain. The nonsense mutation

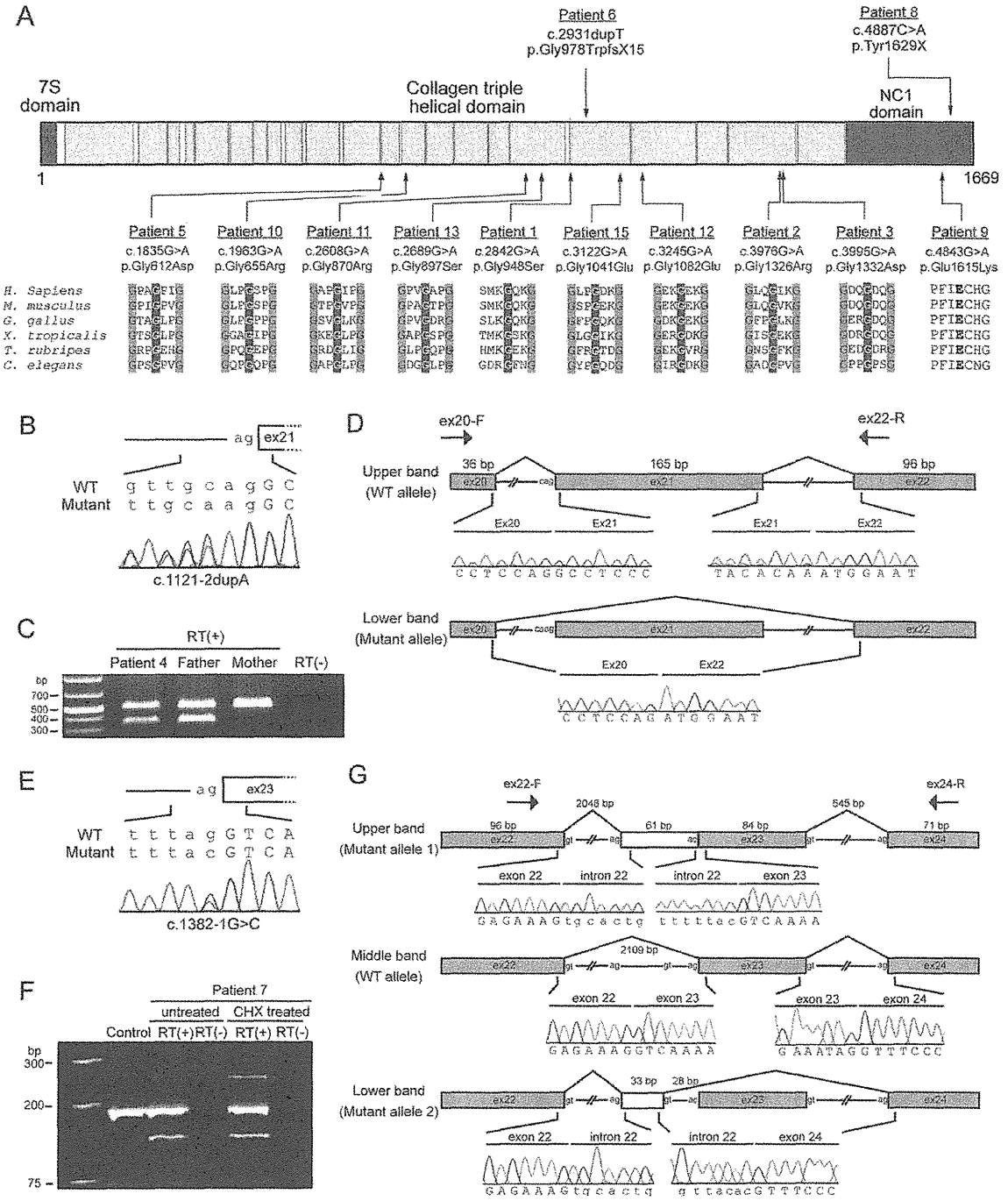

FIGURE 1: *COL4A1* mutations in patients with porencephaly or schizencephaly. (A) Functional domains of COL4A1 protein. The locations of 12 mutations, including 10 missense mutations (bottom), a nonsense mutation, and a frameshift mutation (top) are indicated by arrows. The 7S domain is highlighted with blue and the NC1 domain with red. Gly-X-Y repeats within the collagen triple helical domain are highlighted with yellow. All of the missense mutations occurred at evolutionary conserved amino acids. The positions of the conserved Gly residues in the Gly-X-Y repeats are highlighted in gray. Homologous sequences were aligned using CLUSTALW (http://www.genome.jp/tools/clustalw/). (B) The c.1121-2dupA mutation in intron 20 is colored red. Sequences of exons and introns are presented in upper and lower cases, respectively. (C) Reverse transcriptase (RT)-polymerase chain reaction (PCR) analysis of patient 4 and his parents. (D) Schematic presentation of the wild-type (WT; upper) and mutant (lower) transcripts and primers used for analysis. A single band (500bp), corresponding to the WT allele, was amplified using the mother's cDNA template. Conversely, a lower band was detected from the cDNA from the patient and his father. In the mutant transcript, the 165bp exon 21 was deleted. Sequences of exons and introns are presented in upper and lower cases, respectively. (E) The c.1382-1G>C mutation in intron 22 is colored red. (F) RT-PCR analysis of patient 7 and a control. (G) Schematic representation of the WT and mutant transcripts, and primers used for analysis. A single band (183bp), corresponding to the WT allele, was amplified using a control cDNA template. Conversely, upper and lower bands were detected from the patient's cDNA. The upper band (244bp), which was observed only in cycloheximide (CHX)-treated cells, had a 61bp insertion of intron 22 sequences, leading to a frameshift. Absence of the upper band in untreated lymphoblastoid cell lines strongly suggests that the mutant transcript may undergo nonsense-mediated mRNA decay. The lower band had a 33bp insertion of intron 22 and 84bp deletion of the whole of exon 23, leading to an in-frame 51bp deletion.

TABLE: Clinical features of patients with *COL4A1* mutations

Cases	Age	Sex	Mutation	Inheritance	Brain MRI/ CT findings	CP	Epi	Ocular features	Family history	ID	Hyper-CK	Other
1	14y	M	c.2842G>A (p.Gly948Ser)	de novo	Bilateral POCE, calcification, hemosiderin deposition	Q	+	-	-	+	-	
2	18m	M	c.3976G>A (p.Gly1326Arg)	de novo	Bilateral SCZ, calcification, hemosiderin deposition	Q	+	-	-	+	-	
3	15m	M	c.3995G>A (p.Gly1332Asp)	Absent in mother	Unilateral SCZ, calcification, hemosiderin deposition	H	+	-	-	+	-	
4	6y	M	c.1121-2dupA[1)]	Paternal	Unilateral POCE	H	+	-	-	+	-	FCD
5	2m	F	c.1835G>A (p.Gly612Asp)	ND	Bilateral SCZ, calcification, thin CC, thin brain stem, cerebellar atrophy, absence of SP, hemosiderin deposition, multicystic encephalomalacia,	Q	+	Optic nerve hypoplasia	-	+	+	HA
6	7y	M	c.2931dupT (p.Gly978TrpfsX15)	Paternal	Unilateral POCE	H	+	-	-	+	+	
7	12y	F	c.1382-1G>C[2)]	ND	Unilateral POCE	H	+	-	-	+	+	Myopathy
8	10y	M	c.4887C>A (p.Tyr1629X)	de novo	Unilateral POCE	H	+	-	Hematuria	+	-	
9	3m	F	c.4843G>A (p.Glu1615Lys)	ND	Bilateral POCE, calcification, hypoplastic CC, hemosiderin deposition, thin	Q	+	Microphthalmia Corneal opacity	-	+	-	VSD, HA

TABLE (Continued)

Cases	Age	Sex	Mutation	Inheritance	Brain MRI/ CT findings	CP	Epi	Ocular features	Family history	ID	Hyper-CK	Other
					brain stem, cerebellar atrophy, multicystic encephalomalacia							
10	2y7m	F	c.1963G>A (p.Gly655Arg)	Paternal[3)]	Bilateral POCE	Q	+	-	POCE, Epi	+	-	-
11	1y	F	c.2608G>A (p.Gly870Arg)	ND	Unilateral POCE, calcification	Q	+	Congenital cataract	-	+	-	-
12	1y5m	M	c.3245G>A (p.Gly1082Glu)	ND	Unilateral SCZ with bilateral POCE, calcification, cerebellar hypoplasia	T	+	Congenital cataract	-	+	-	HA, Hematuria
13	3y7m	M	c.2689G>A (p.Gly897Ser)	de novo	Unilateral POCE	Q	+	-	-	+	+	-
14	9m	F	c.1990+1G>A	de novo	Unilateral POCE, hemosiderin deposition	Q	+	-	-	+	+	HA, Hematuria
15	2y	F	c.3122G>A (p.Gly1041Glu)	ND	Unilateral SCZ, hemosiderin deposition	Q	+	-	-	+	+	HA

1) p.Gly374_Asn429delinsAsp change was predicted by mRNA analysis
2) Two alternative protein chages were predicted by mRNA analysis: p.Gly461_Gly489delinsValHisCysGlyAspPheTrpSerHisValThrArg and p.Gly461ValfsX31. y, years; m, months; M, male; F, female; ND, Not determined; POCE, porencephaly, SCZ, schizencephaly; CC, corpus callosum; SP, septum pellucidum; CP, cerebral palsy; H, hemiplegia; T, Triplegia; Q, quadriplegia; Epi, epilepsy; ID, intellectual disability; CK, creatine kinase; FCD, Focal cortical dysplasia; HA, Hemolytic anemia; VSD, ventricular septal defect
3) Co-segregation of the p.Gly655Arg mutation with porencephaly was confirmed.

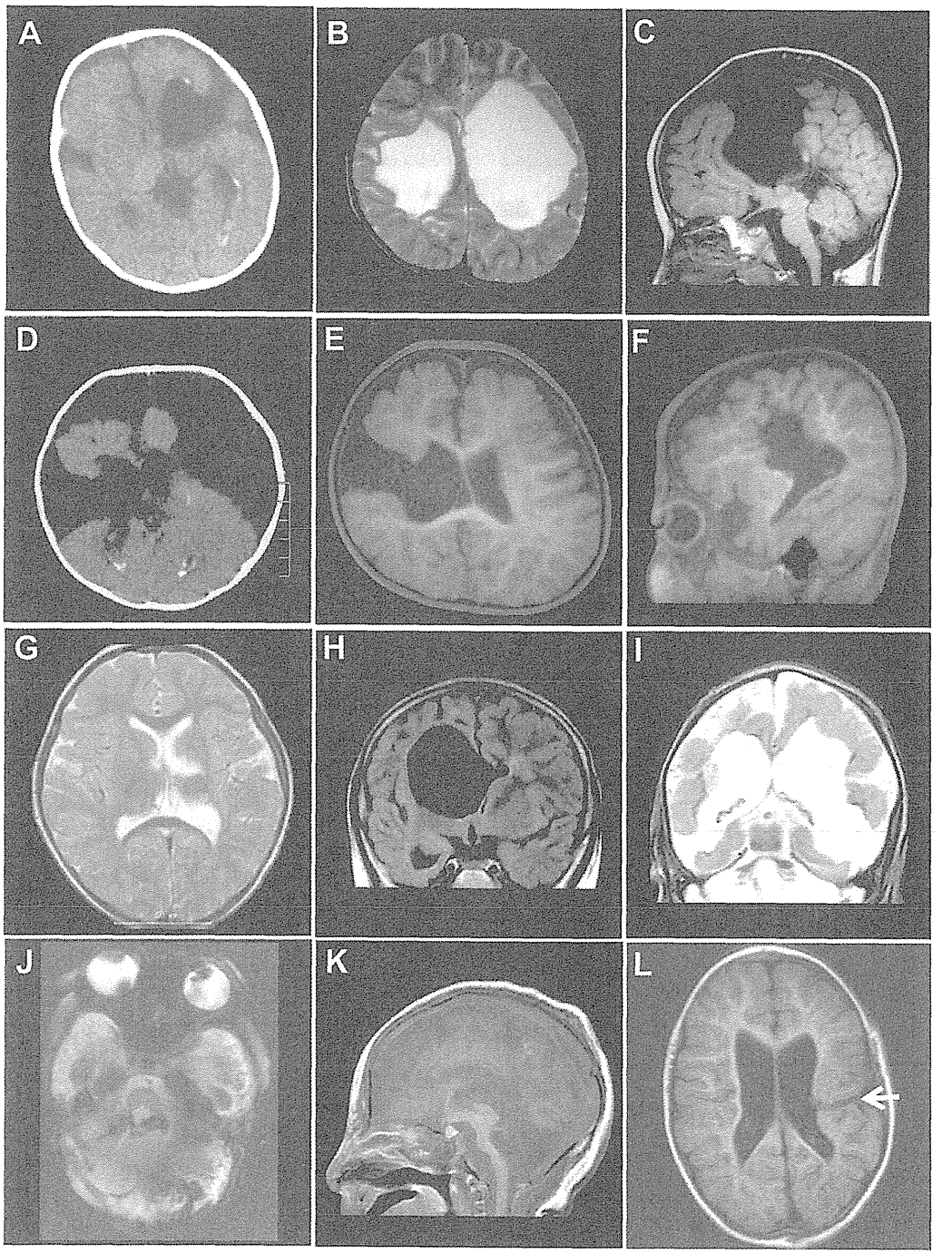

FIGURE 2: Computed tomography (CT) scan (A, D) and magnetic resonance imaging (MRI; B, C, E–L) of patients with *COL4A1* mutations. (A–C) Images of patient 1. (A) The CT scan shows calcification along with the dilated lateral ventricular wall. (B) T2-weighted and (C) T1-weighted images (WIs) at 5 years of age showing bilateral porencephaly. (D) The CT image of patient 2 with schizencephaly shows calcification of the lateral ventricular wall and brain parenchyma. (E, F) T1-WIs of patient 3 show unilateral schizencephaly at 15 months of age. (G) T2-WI of patient 4 at 3 years of age shows parenchymal defect of the left thalamus and basal ganglia due to subependymal hemorrhage. (H) Fluid-attenuated inversion recovery image of patient 7 at 6 years of age showing unilateral porencephaly. (I) T2-WI, (J) T2*-weighted gradient-echo image (WGRE), and (K) T1-WI of patient 9. (I) The MRI at 2 months of age shows bilateral porencephaly with low-intensity lesions along with a deformed ventricular wall, which has hemosiderin deposition and calcification. (J) T2*-WGRE showing hemosiderin deposition in the atrophic cerebellum. The atrophic pontocerebellar structures are also shown in (K). (L) T1-WI of patient 15 showed schizencephaly in the left hemisphere at 2 years of age.

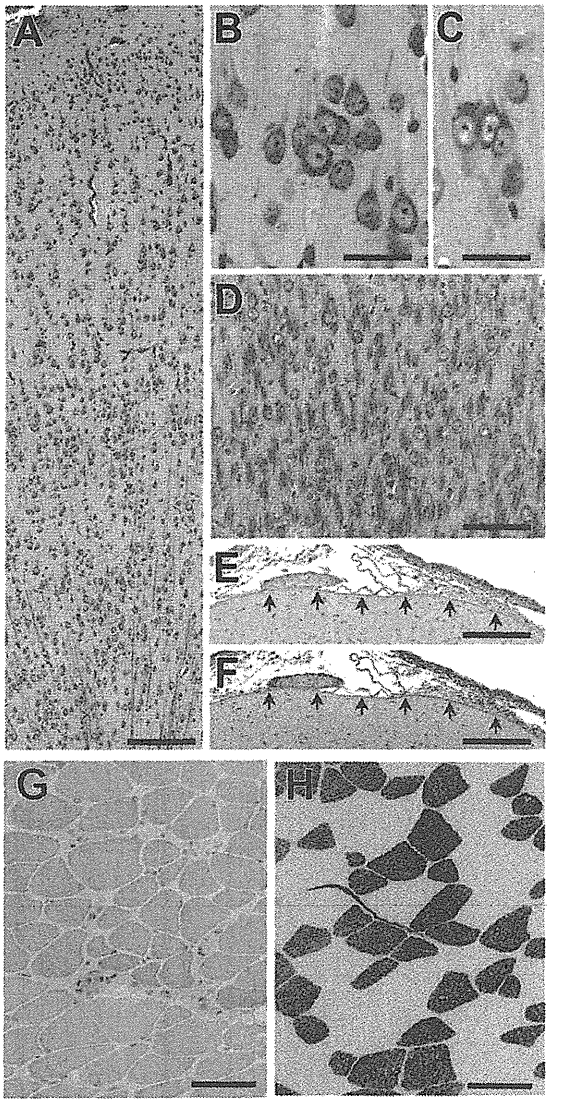

FIGURE 3: Histopathological features of the resected frontal tissue of patient 4 (A–F) and biopsied rectus abdominis muscle of patient 7 (G, H). (A) Low-magnification view of the cortex showing architectural abnormalities. (B, C) Two examples of neuronal clustering. (D) Many neurons scattered within the subcortical white matter. (E, F) Two serial sections demonstrating the superficial layer of the cortex. Note that the basal lamina of the pia mater (*arrows* in each panel) is continuously labeled with antibodies against collagen type IV (E) and laminin (F). (A–D) Klüver–Barrera stain. (E, F) Immunostained and then counterstained with hematoxylin. (G) Hematoxylin and eosin staining showing variation in fiber size, slightly increased endomysial connective tissue, and internal nuclei. (H) Adenosine triphosphatase (pH 4.5) staining showing type 2B fiber deficiency. There was no increase in number of type 2C fibers. Scale bars indicate 175μm (A, E, F), 30μm (B, C), 80μm (D), and 30μm (G, H).

would cause a truncation of the NC1 domain rather than mRNA degradation by NMD as the mutation was located within 50bp of the exon–intron boundary of the second to last exon (exon 51).[26] The NC1 domains are the sites for molecular recognition through which the stoichiometry of chains in the assembly of triple-helical formation is directed[1]; therefore, these 2 mutations may alter the assembly of the collagen IV $\alpha1\alpha1\alpha2$ heterotrimers. In addition, the effect of 2 splice site mutations was examined using LCL, suggesting that in-frame deletion/insertion mutant protein should be produced. Thus, it is highly likely that impairment of the collagen IV $\alpha1\alpha1\alpha2$ heterotrimer assembly caused by mutant $\alpha1$ chain is a common pathological mechanism of *COL4A1* mutations. The c.2931dupT mutation found in patient 6 and his father might cause severe truncation of COL4A1 protein. It is possible that the truncation of COL4A1 protein can also impair $\alpha1\alpha1\alpha2$ heterotrimer assembly similar to substitutions of conserved Gly residues in the Gly-X-Y repeat. Alternatively, the mutant transcript might undergo NMD, and haploinsufficiency of *COL4A1* might cause a weakness of basement membrane. Biological analysis using patients' cells will clarify these possibilities.

COL4A1 mutations in schizencephaly were first demonstrated in this study. Schizencephaly was used by Yakovlev and Wadsworth in 1946 to describe true clefts formed in the brain as a result of failure of development of the cortical mantle in the zones of cleavage of the primary cerebral fissures.[19] Schizencephaly is differentiated from clefts in the central mantle that arise as the result of a destruction of the cerebral tissues, which they called encephalocrastic porencephalies, now known simply as porencephaly.[19] Schizencephaly has been understood as a neuronal migration disorder, because the clefts are lined by abnormal gray matter, described as polymicrogyria. Conversely, porencephaly is understood to be a postmigration accident resulting in lesions, without gray matter lining the clefts or an associated malformation of cortical development. It has been suggested that both schizencephaly and porencephaly are caused by encephaloclastic regions, and can be distinguished depending on time of insult.[16,17] The present study clearly demonstrated that *COL4A1* mutations caused both porencephaly and schizencephaly, supporting the same pathological mechanism for these 2 conditions.

The genes responsible for FCD have been elusive, despite extensive investigation. The pathological features of the cortical tubers of tuberous sclerosis (TSC) may be indistinguishable from those of FCD. Apart from FCD due to TSC, there is only 1 gene that may explain the genetic basis of FCD, where a homozygous mutation in *CNTNAP2* has been identified in Amish children with FCD, macrocephaly, and intractable seizures.[27] Surprisingly, the present study discovered a patient with FCD

and porencephaly, in whom aberrant splicing was demonstrated and FCD1A was pathologically confirmed using resected brain tissues. A recent report revealed *COL4A1* mutations in 2 patients with MEB/WWS showing cobblestone lissencephaly,[15] and abnormal cortical development has been observed in mouse models of *COL4A1* mutations.[15,28] Thus, it is possible that *COL4A1* mutations are involved in cerebral cortical malformations, including FCD. Identification of a greater number of cases is required to confirm the association between *COL4A1* mutations and cortical malformations in humans.

In a few children, the sequelae were much more severe than would be expected on the basis of their imaging findings. This is of importance when counseling parents with regard to prediction of neurodevelopmental outcome.

Two patients with *COL4A1* mutations showed intracranial calcification, pontocerebellar atrophy, ocular abnormalities, and hemolytic anemia associated with severe bilateral porencephaly (patient 9) or schizencephaly (patient 5). Severe hemorrhagic destructive lesions in the cerebrum were observed in these patients, and T2* images also showed hemorrhage in the cerebellum, which may have resulted in a thin brainstem and severe cerebellar atrophy. Thus, these 2 patients could be considered as the most severe manifestations affecting the developing brain and eyes. A common feature of the 2 patients is hemolytic anemia of an unknown cause, which required frequent blood transfusions. Five of 15 patients with *COL4A1* mutations showed hemolytic anemia. Interestingly, 2 reports have demonstrated that mouse *Col4a1* mutants showed a significant reduction in red blood cell (RBC) number and hematocrit.[28,29] Given that *Col4a1* mutations lead to hemorrhage, chronic hemorrhage is possibly involved in RBC loss. Alternatively, the *Col4a1* mutation may directly affect blood progenitor cells, as they transmigrate across basement membranes before entering the peripheral blood.[30] Hemolytic anemia in patients with *COL4A1* mutations would imply the latter explanation. Further studies are required to clarify how *COL4A1/Col4a1* mutations are involved in anemia.

In summary, we found 15 mutations in *COL4A1* among 71 patients with porencephaly or schizencephaly, showing an unexpectedly high percentage of mutations (about 21%) in these patients. Fourteen patients with *COL4A1* mutations had no family history of cerebral palsy. The 15 patients with *COL4A1* mutations showed a variety of phenotypes, further expanding the possible clinical spectrum of *COL4A1* mutations to include schizencephaly, FCD, pontocerebellar atrophy, and hemolytic anemia. Genetic testing for *COL4A1* should be recommended for children with porencephaly and schizencephaly.

Acknowledgment

This work was supported by research grants from the Ministry of Health, Labor, and Welfare (K.H., M.K., H.O., N.Mi., N.Ma., H.S.), Japan Science and Technology Agency (N.Ma.), and Strategic Research Program for Brain Sciences (11105137 to N.Ma.), a Grant-in-Aid for Scientific Research on Innovative Areas (Foundation of Synapse and Neurocircuit Pathology) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan (11001956 to N.Ma.), a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (H.O., N.Ma.), a Grant-in-Aid for Young Scientists from the Japan Society for the Promotion of Science (H.D., N.Mi., H.S.), and a grant from the Takeda Science Foundation (N.Mi., N.Ma.). This work was performed at the Advanced Medical Research Center, Yokohama City University, Japan.

We thank the patients and their family members for their participation in this study.

Authorship

Y.Y. and K.H. contributed equally to this work.

Potential Conflicts of Interest

Nothing to report.

References

1. Khoshnoodi J, Pedchenko V, Hudson BG. Mammalian collagen IV. Microsc Res Tech 2008;71:357–370.
2. Gould DB, Phalan FC, Breedveld GJ, et al. Mutations in Col4a1 cause perinatal cerebral hemorrhage and porencephaly. Science 2005;308:1167–1171.
3. Breedveld G, de Coo IF, Lequin MH, et al. Novel mutations in three families confirm a major role of COL4A1 in hereditary porencephaly. J Med Genet 2006;43:490–495.
4. Yoneda Y, Haginoya K, Arai H, et al. De novo and inherited mutations in COL4A2, encoding the type IV collagen alpha2 chain cause porencephaly. Am J Hum Genet 2012;90:86–90.
5. Vahedi K, Alamowitch S. Clinical spectrum of type IV collagen (COL4A1) mutations: a novel genetic multisystem disease. Curr Opin Neurol 2011;24:63–68.
6. Lanfranconi S, Markus HS. COL4A1 mutations as a monogenic cause of cerebral small vessel disease: a systematic review. Stroke 2010;41:e513–e518.
7. van der Knaap MS, Smit LM, Barkhof F, et al. Neonatal porencephaly and adult stroke related to mutations in collagen IV A1. Ann Neurol 2006;59:504–511.
8. Meuwissen ME, de Vries LS, Verbeek HA, et al. Sporadic COL4A1 mutations with extensive prenatal porencephaly resembling hydranencephaly. Neurology 2011;76:844–846.

9. Livingston J, Doherty D, Orcesi S, et al. COL4A1 Mutations associated with a characteristic pattern of intracranial calcification. Neuropediatrics 2011;42:227–233.
10. Plaisier E, Gribouval O, Alamowitch S, et al. COL4A1 mutations and hereditary angiopathy, nephropathy, aneurysms, and muscle cramps. N Engl J Med 2007;357:2687–2695.
11. Alamowitch S, Plaisier E, Favrole P, et al. Cerebrovascular disease related to COL4A1 mutations in HANAC syndrome. Neurology 2009;73:1873–1882.
12. Coutts SB, Matysiak-Scholze U, Kohlhase J, Innes AM. Intracerebral hemorrhage in a young man. CMAJ 2011;183:E61–E64.
13. Sibon I, Coupry I, Menegon P, et al. COL4A1 mutation in Axenfeld-Rieger anomaly with leukoencephalopathy and stroke. Ann Neurol 2007;62:177–184.
14. Weng YC, Sonni A, Labelle-Dumais C, et al. COL4A1 mutations in patients with sporadic late-onset intracerebral hemorrhage. Ann Neurol 2012;71:470–477.
15. Labelle-Dumais C, Dilworth DJ, Harrington EP, et al. COL4A1 mutations cause ocular dysgenesis, neuronal localization defects, and myopathy in mice and Walker-Warburg syndrome in humans. PLoS Genet 2011;7:e1002062.
16. Friede R. Porencephaly, hydranencephaly, multicystic encephalopathy. In: Developmental neuropathology. 2nd ed. Berlin, Germany: Springer-Verlag, 1989:28–43.
17. Govaert P. Prenatal stroke. Semin Fetal Neonatal Med 2009;14: 250–266.
18. Barkovich AJ, Guerrini R, Kuzniecky RI, et al. A developmental and genetic classification for malformations of cortical development: update 2012. Brain 2012;135:1348–1369.
19. Yakovlev PI, Wadsworth RC. Schizencephalies; a study of the congenital clefts in the cerebral mantle; clefts with fused lips. J Neuropathol Exp Neurol 1946;5:116–130.
20. Barkovich AJ, Kjos BO. Schizencephaly: correlation of clinical findings with MR characteristics. AJNR Am J Neuroradiol 1992;13: 85–94.
21. Saitsu H, Kato M, Mizuguchi T, et al. De novo mutations in the gene encoding STXBP1 (MUNC18–1) cause early infantile epileptic encephalopathy. Nat Genet 2008;40:782–788.
22. Saitsu H, Kato M, Okada I, et al. STXBP1 mutations in early infantile epileptic encephalopathy with suppression-burst pattern. Epilepsia 2010;51:2397–2405.
23. Lichtenbelt KD, Pistorius LR, De Tollenaer SM, et al. Prenatal genetic confirmation of a COL4A1 mutation presenting with sonographic fetal intracranial hemorrhage. Ultrasound Obstet Gynecol 2012;39:726–727.
24. de Vries LS, Koopman C, Groenendaal F, et al. COL4A1 mutation in two preterm siblings with antenatal onset of parenchymal hemorrhage. Ann Neurol 2009;65:12–18.
25. Engel J, Prockop DJ. The zipper-like folding of collagen triple helices and the effects of mutations that disrupt the zipper. Annu Rev Biophys Biophys Chem 1991;20:137–152.
26. Nagy E, Maquat LE. A rule for termination-codon position within intron-containing genes: when nonsense affects RNA abundance. Trends Biochem Sci 1998;23:198–199.
27. Strauss KA, Puffenberger EG, Huentelman MJ, et al. Recessive symptomatic focal epilepsy and mutant contactin-associated protein-like 2. N Engl J Med 2006;354:1370–1377.
28. Favor J, Gloeckner CJ, Janik D, et al. Type IV procollagen missense mutations associated with defects of the eye, vascular stability, the brain, kidney function and embryonic or postnatal viability in the mouse, Mus musculus: an extension of the Col4a1 allelic series and the identification of the first two Col4a2 mutant alleles. Genetics 2007;175:725–736.
29. Van Agtmael T, Bailey MA, Schlotzer-Schrehardt U, et al. Col4a1 mutation in mice causes defects in vascular function and low blood pressure associated with reduced red blood cell volume. Hum Mol Genet 2010;19:1119–1128.
30. Janowska-Wieczorek A, Marquez LA, Nabholtz JM, et al. Growth factors and cytokines upregulate gelatinase expression in bone marrow CD34(+) cells and their transmigration through reconstituted basement membrane. Blood 1999;93:3379–3390

Journal of Child Neurology
000(00) 1-4

DOI: 10.1177/0883073811416239
http://jcn.sagepub.com
SAGE

Congenital Dysplastic Microcephaly and Hypoplasia of the Brainstem and Cerebellum With Diffuse Intracranial Calcification

Kazuyuki Nakamura, MD, Mitsuhiro Kato, MD, PhD, Ayako Sasaki, MD, PhD, Masayo Kanai, MD, PhD, and Kiyoshi Hayasaka, MD, PhD

Abstract

Congenital microcephaly with intracranial calcification is a rare condition presented in heterogeneous diseases. Here, we report the case of a 1-year-old boy with severe congenital microcephaly and diffuse calcification. Neuroimaging studies showed a diffuse simplified gyral pattern; a very thin cortex; ventricular dilatation; very small basal ganglia, thalamus, and brainstem; and cerebellar hypoplasia with diffuse calcification. Clinical features of intrauterine infections, such as neonatal jaundice, hepatomegaly, and thrombocytopenia, were not found. Serological tests, cultures, and polymerase chain reaction analysis were negative for viral infections. The etiology of pseudo–toxoplasmosis, rubella, cytomegalovirus, and herpes simplex syndrome is still unknown. This study describes the most severe form of pseudo–toxoplasmosis, rubella, cytomegalovirus, and herpes simplex syndrome reported to date, with the patient showing microcephaly and calcification or band-like intracranial calcification with simplified gyration and polymirogyria.

Keywords

microcephaly, intracranial calcification, pontocerebellar hypoplasia, toxoplasmosis, rubella, cytomegalovirus, herpes simplex

Received February 23, 2011. Accepted for publication June 16, 2011.

Congenital microcephaly with brain dysgenesis and intracranial calcification is a characteristic feature of intrauterine infections of toxoplasma, rubella, cytomegalovirus, herpes virus, and other infectious agents, including human immunodeficiency virus and the bacteria that cause syphilis. This form of congenital microcephaly has been termed the toxoplasmosis, rubella, cytomegalovirus, and herpes simplex syndrome.[1] In addition to congenital microcephaly and intracranial calcification, toxoplasmosis, rubella, cytomegalovirus, and herpes simplex syndrome shows systemic abnormalities, such as thrombocytopenia, anemia, hepatosplenomegaly, liver dysfunction, jaundice, and chorioretinitis, with elevated serum immunoglobulin M (IgM) levels at birth. Similar clinical conditions have been reported in several patients with familial occurrence but with no evidence of infection. These clinical conditions have been designated as "pseudo–toxoplasmosis, rubella, cytomegalovirus, and herpes simplex" syndrome.[2] In addition, "band-like intracranial calcification with simplified gyration and polymicrogyria" has also been reported. However, this syndrome shows no evidence of infection, abnormalities in liver function, or thrombocytopenia.[3,4]

It is important to discriminate these syndromes for genetic counseling. This report describes a patient with congenital microcephaly and whole brain dysgenesis and extensive calcification, suggesting a severe form of pseudo–toxoplasmosis, rubella, cytomegalovirus, and herpes simplex syndrome, or band-like intracranial calcification with simplified gyration and polymicrogyria.

Case Report

The boy was born to healthy, unrelated, 29-year-old Japanese parents. This was the mother's first pregnancy, and the boy had no siblings. There were no household pets including cats. During the 5- to 6-week period of gestation, his mother had fever for 1 day but showed no other symptoms. Microcephaly was first observed on ultrasound examination conducted at 28 weeks of gestation. At 34 weeks of gestation, specific IgMs

Department of Pediatrics, Yamagata University Faculty of Medicine, Yamagata, Japan

Corresponding Author:
Kazuyuki Nakamura, MD, Department of Pediatrics, Yamagata University Faculty of Medicine, 2-2-2, Iida-Nishi, Yamagata 990-9585, Japan
Email: kazun-yamagata@umin.ac.jp

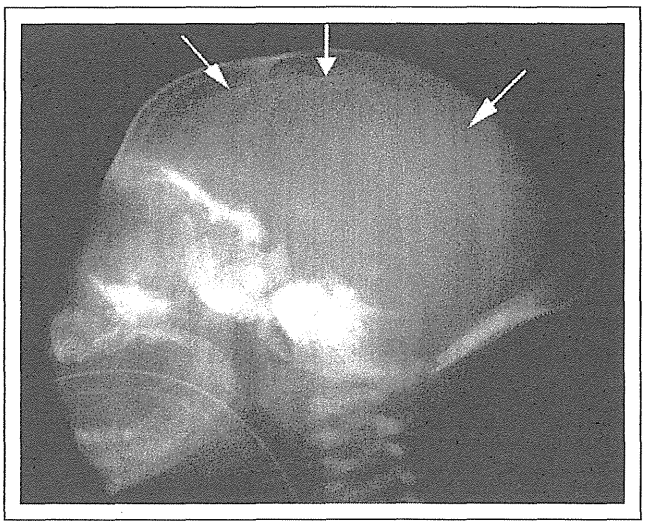

Figure 1. A plain radiograph of the head shows the cranial vault with a frontal sloping and a marked external occipital protuberance. Intracranial high-density spots are visible along the cerebral wall (arrows).

against rubella, cytomegalovirus, and toxoplasma were negative in the mother. He was delivered by caesarean section because of hypotonic uterine dysfunction at 42 weeks of gestation. His Apgar score was 1 at 1 min. He required intratracheal intubation for severe dyspnea. His weight at birth was 3140 g; length, 46 cm (−2.0 SD); and head circumference, 29 cm (−3.2 SD). He had bilateral undescended testis without hepatosplenomegaly or a petechial rash. Ophthalmologic examination showed no corneal clouding or chorioretinitis. Neurological examination showed the presence of hypotonic muscles and absence of a Moro reflex. Skull radiography showed a sloping forehead and several intracranial calcific densities (Fig. 1). A computed tomographic (CT) scan of the head showed prominent calcification mainly along the ventricular wall from the cerebrum to the brain stem (Fig. 2). Magnetic resonance imaging (MRI) of the brain showed severe diffuse simplified gyri combined with a thinning of the cortex and the white matter; marked dilated ventricles; and severe hypoplasia of the basal ganglia, thalamus, cerebellum, and brainstem (Fig. 3). At the age of 4 days, a hematological examination (hemoglobin, 18.3 g/dL; white blood cells, 10 010/μL; neutrophils, 69%; platelets, 32.8 $\times$ $10^4/\mu L^3$) and blood chemistry tests, including those for calcium, phosphate, aspartate aminotransferase, alanine aminotransferase, lactate, and amino acids were normal. The total serum IgM was 8 mg/dL; the specific IgMs against toxoplasma, rubella, cytomegalovirus, herpes simplex, and varicella-zoster virus were negative. Viral cultures of a pharyngeal swab and urine were negative. Subsequent polymerase chain reaction (PCR) amplification of cytomegalovirus DNA in urine and the umbilical cord was also negative. An antibody titer for lymphocytic choriomeningitis virus was negative. G-banding chromosomal analysis showed a karyotype of 46, XY. At the age of 10 months, a cerebrospinal fluid examination did not show increased number of lymphocytes or interferon-alpha.

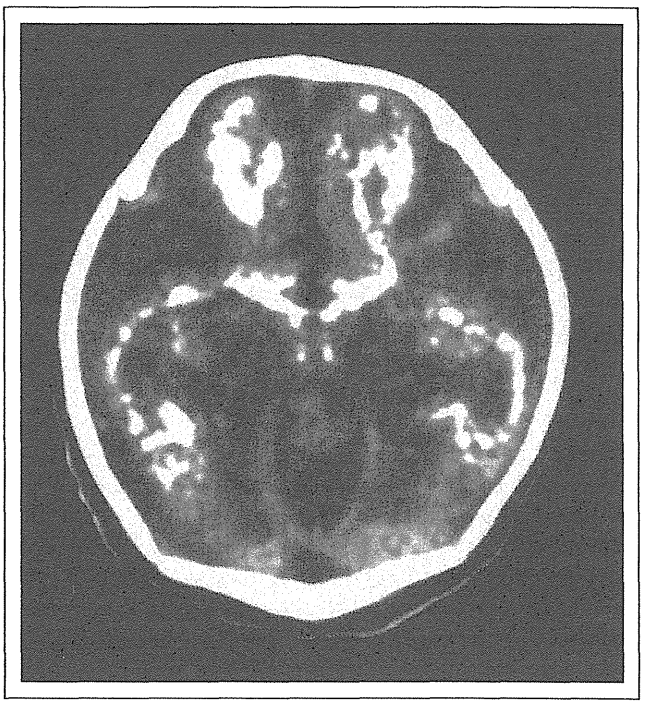

Figure 2. Computed tomographic scan of the head shows linear or patchy high signals consisting of calcifications within or immediately beneath the cortex and the enlargement of the lateral ventricles.

Electroencephalography showed multifocal sharp waves with low-voltage background activity. At 12 months of age, he showed hypothermia (<36°C); recurrent urinary tract infections caused by vesicoureteral reflux; and a profound developmental delay with limb contractures, no eye contact, and no head control. He received tube feeding because of bulbar palsy.

Discussion

Congenital microcephaly results from various clinical conditions such as infections, radiation, exogenous toxic agents, anoxic or metabolic insults, and genetic bases.[1] Although these conditions can also cause cerebral calcification, the association of congenital microcephaly and calcification are rare. Our patient experienced a febrile episode, suggesting an infection at 5 to 6 weeks of gestation, but there was no history of any other insult. Since intrauterine infections or toxoplasmosis, rubella, cytomegalovirus, and herpes simplex syndrome, especially cytomegalovirus infection, is the most frequent cause of congenital microcephaly and since calcification and earlier infection induce severer brain anomalies,[1] we tried to obtain evidence of a prenatal infection of cytomegalovirus using serological tests, cultures, and PCR amplification. However, none of these tests yielded a positive result, which could be a result of early infection during fetal development. The fetus showed no immunological response, and the virus could not be cultured. Moreover, the PCR sensitivity was sufficiently high enough to detect viral DNA.[5] On

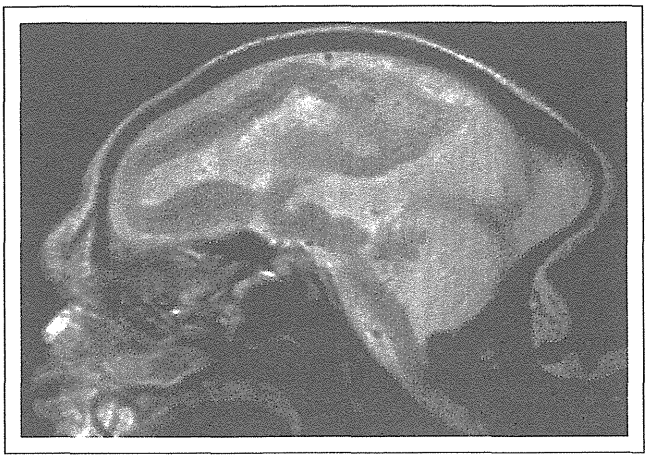

Figure 3. Sagittal T2-weighted magnetic resonance imaging (MRI) shows a thin cortex and white matter with an undetectable border. Prominent hypoplasia of the cerebellum and brainstem is visible.

the basis of these results, we conclude that cytomegalovirus is unlikely to be the cause of our patient's condition.

During the middle embryonic period (5–6 weeks of gestation in humans), secondary brain vesicles (telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon) are formed; the primitive cerebral hemispheres develop during neuronogenesis in the ventricular zone. Insult at this stage can engender extremely severe brain malformation (eg, decreased brain size) because of the inhibition of cell proliferation and the dysplastic configuration of the brain that results from impaired cell migration. The hypoplastic brainstem and cerebellum as well as microcephaly with the thin cortex and irregular convolution seen in our patient suggest an event during early embryogenesis, although the etiology is unknown.

Although our patient showed an irregular convolution that suggested possible cortical dysplasia, the very thin cortex suggested microcephaly with normal to thin cortex or microcephaly with a simplified gyral pattern.[6] To date, 5 genes (*MCPH1, ASPM, CDK5RAP2, CENPJ,* and *SLC25A19*) have been found to be responsible for the autosomal recessive inheritance of microcephaly with a simplified gyral pattern.[7,8] Mutations in these genes result in congenital microcephaly but not in a calcification resembling the one in our patient. Microcephaly with polymicrogyria or other cortical dysplasias or microcephaly with pontocerebellar hypoplasia are other candidate conditions for our patient, but neither one shows calcification.[9] At this point, it is difficult to categorize the neuroimaging features of our patient into the classification scheme for malformations of cortical development.[6]

Aicardi–Goutières syndrome is an autosomal recessive form of progressive encephalopathy characterized by acquired microcephaly, leukodystrophy, and calcifications of the basal ganglia, which is similar to toxoplasmosis, rubella, cytomegalovirus, and herpes simplex syndrome.[10] Recently, 5 genes (*TREX1, RNASEH2B, RNASEH2C, RNASEH2A,* and *SAMHD1*) have been identified as the responsible genes for this syndrome.[11-13] Elevated interferon-alpha levels and chronic lymphocytosis in the cerebrospinal fluid are specific features of Aicardi–Goutières syndrome. However, our patient had neither of these features. Although cerebrospinal fluid lymphocytosis is not necessary for an Aicardi–Goutières syndrome diagnosis[14] and although a small number of patients show microcephaly at birth, the cerebral dysplasia observed in our patient has never been reported as a manifestation of Aicardi–Goutières syndrome.

It has been suggested that pseudo–toxoplasmosis, rubella, cytomegalovirus, and herpes simplex syndrome is the same disorder as Aicardi–Goutières syndrome.[15] Periventricular areas are commonly calcified in pseudo–toxoplasmosis, rubella, cytomegalovirus, and herpes simplex syndrome, but the basal ganglia, cerebellum, and brainstem can also be affected. Brain MRIs often show cerebral atrophy, enlarged lateral ventricles, and severe hypoplasia of the corpus callosum, cerebellum, and brainstem. Some patients also show associated cortical dysplasia.[2,16] One report described a patient as having microcephaly with plate-like cortical calcification and with an extremely decreased convolution of the cerebral cortex, which is similar to our patient's condition.[17] However, the brainstem and cerebellum were spared in that patient.

Band-like intracranial calcification with simplified gyration and polymicrogyria is inherited as an autosomal recessive trait, and mutations of the *OCLN* gene have been identified as resulting in this condition.[18] Band-like intracranial calcification with simplified gyration and polymicrogyria is similar to pseudo–toxoplasmosis, rubella, cytomegalovirus, and herpes simplex syndrome in that both show widespread intracranial calcification and polymicrogyria and that some patients show hypoplasia of the cerebellum and brainstem. There are differences between these 2 conditions in terms of the postnatal microcephaly, the characteristic band-like calcification, and the lack of evidence for neonatal disturbance of liver function with thrombocytopenia; nonetheless, they can have similar etiologies.[3,4]

The extensive lesions of the brain are reminiscent of multicystic encephalomalacia, which are often accompanied by extensive dystrophic calcifications in zones of infarction. Multicystic encephalomalacia is also caused by fetal viral infection as well as hypoxia or circulatory insults; however, the lesions in the patient appeared to be too broad for secondary injury and had no visible cysts, as observed by MRI. The size and number of cysts depends on the stage of infarction, which can be both of major cerebral vessels and of the microcirculation at capillary levels.[19] Neuropathological confirmation is essential to reveal the pathogenesis.

It is noteworthy that this is the most severe case of a patient with congenital dysplastic microcephaly and brainstem and cerebellar hypoplasia with extensive intracranial calcification. The pathogenesis, particularly regarding its inheritance, remains to be clarified.

Acknowledgments

The authors thank Dr William B. Dobyns of the University of Chicago for his valuable comments and Dr Keiko Ishii, Tohoku University, for examining cytomegalovirus infection.

Author Contributions

KN contributed in organizing the article and wrote the first draft of the manuscript. M. Kato and KH performed a review and critique of the manuscript. KN, M. Kato, AS, and M. Kanai primarily managed the patient.

Declaration of Conflicting Interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

Ethical Approval

The authors received an informed consent form from the parents of the patient.

References

1. Volpe JJ. In: *Neurology of the Newborn*. 5th ed. Philadelphia, PA: WB Saunders;; 2008.
2. Vivarelli R, Grosso S, Cioni M, et al. Pseudo-TORCH syndrome or Baraitser-Reardon syndrome: diagnostic criteria. *Brain Dev*. 2001;23:18-23.
3. Briggs TA, Wolf NI, D'Arrigo S, et al. Band-like intracranial calcification with simplified gyration and polymicrogyria: a distinct "pseudo-TORCH" phenotype. *Am J Med Genet A*. 2008;146: 3173-3180.
4. Abdel-Salam GM, Zaki MS, Saleem SN, Gaber KR. Microcephaly, malformation of brain development and intracranial calcification in sibs: pseudo-TORCH or a new syndrome. *Am J Med Genet A*. 2008;146:2929-2936.
5. Revello MG, Zavattoni M, Furione M, et al. Diagnosis and outcome of preconceptional and periconceptional primary human cytomegalovirus infections. *J Infect Dis*. 2002;186:553-557.
6. Barkovich AJ, Kuzniecky RI, Jackson GD, et al. A development and genetic classification for malformations of cortical development. *Neurology*. 2005;65:1873-1887.
7. Rosenberg MJ, Agarwala R, Bouffard G, et al. Mutant deoxynucleotide carrier is associated with congenital microcephaly. *Nat Genet*. 2002;32:175-179.
8. Woods CG, Bond J, Enard W. Autosomal recessive primary microcephaly (MCPH): a review of clinical, molecular, evolutionary findings. *Am J Hum Genet*. 2005;76:717-728.
9. Hashimoto K, Takeuchi Y, Kida Y, et al. Three siblings of fatal infantile encephalopathy with olivopontocerebellar hypoplasia and microcephaly. *Brain Dev*. 1998;20:169-174.
10. Goutières F. Aicardi-Goutières syndrome. *Brain Dev*. 2005;27: 201-206.
11. Crow YJ, Leitch A, Hayward BE, et al. Mutations in genes encoding ribonuclease H2 subunits cause Aicardi-Goutières syndrome and mimic congenital viral brain infection. *Nat Genet*. 2006;38: 910-916.
12. Crow YJ, Hayward BE, Parmar R, et al. Mutations in the gene encoding the 3'-5' DNA exonuclease TREX1 cause Aicardi–Goutières syndrome at the AGS1 locus. *Nat Genet*. 2006;38: 917-920.
13. Rice GL, Bond J, Asipu A, et al. Mutations involved in Aicardi–Goutières syndrome implicate SAMHD1 as regulator of the innate immune response. *Nat Genet*. 2009;41:829-832.
14. Crow YJ, Black DN, Ali M, et al. Cree encephalitis is allelic with Aicardi-Goutières syndrome: implications for the pathogenesis of disorders of interferon alpha metabolism. *J Med Genet*. 2003;40: 183-187.
15. Sanchis A, Cerveró L, Bataller A, et al. Genetic syndromes mimic congenital infections. *J Pediatr*. 2005;146:701-705.
16. Burn J, Wickramasinghe HT, Harding B, Baraitser M. A syndrome with intracranial calcification and microcephaly in two sibs, resembling intrauterine infection. *Clin Genet*. 1986; 30:112-116.
17. Kalyanasundaram S, Dutta S, Narang A, Katariya S. Microcephaly with plate-like cortical calcification. *Brain Dev*. 2003;25: 130-132.
18. O'Driscoll MC, Daly SB, Urquhart JE, et al. Recessive mutations in the gene encoding the tight junction protein occludin cause band-like calcification with simplified gyration and polymicrogyria. *Am J Hum Genet*. 2010;87:354-364.
19. Weiss JL, Cleary-Goldman J, Tanji K, et al. Multicystic encephalomalacia after first-trimester intrauterine fetal death in monochorionic twins. *Am J Obstet Gynecl*. 2004;190: 563-565.

ELSEVIER

Brain & Development xxx (2013) xxx–xxx

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

www.elsevier.com/locate/braindev

Original article

Magnetoencephalography localizing spike sources of atypical benign partial epilepsy

Hideaki Shiraishi [a], Kazuhiro Haginoya [b], Eiji Nakagawa [c], Shinji Saitoh [a], Yutaka Kaneko [d], Nobukazu Nakasato [e], Derrick Chan [f,g], Hiroshi Otsubo [f,*]

[a] *Department of Pediatrics, Hokkaido University Graduate School of Medicine, Sapporo, Hokkaido, Japan*
[b] *Department of Pediatrics, Tohoku University School of Medicine, Sendai, Miyagi, Japan*
[c] *Department of Neurology, National Hospital for Mental, Nervous and Muscular Disorders, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan*
[d] *Department of Neurosurgery, National Hospital for Mental, Nervous and Muscular Disorders, National Center of Neurology and Psychiatry, Kodaira, Tokyo, Japan*
[e] *Department of Epileptology, Tohoku University School of Medicine, Sendai, Miyagi, Japan*
[f] *Division of Neurology, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada*
[g] *Neurology Service, Department of Pediatric Medicine, KK Women's and Children's Hospital, Singapore*

Received 25 September 2012; received in revised form 24 December 2012; accepted 25 December 2012

Abstract

Rationale: Atypical benign partial epilepsy (ABPE) is characterized by centro-temporal electroencephalography (EEG) spikes, continuous spike and waves during sleep (CSWS), and multiple seizure types including epileptic negative myoclonus (ENM), but not tonic seizures. This study evaluated the localization of magnetoencephalography (MEG) spike sources (MEGSSs) to investigate the clinical features and mechanism underlying ABPE. *Methods:* We retrospectively analyzed seizure profiles, scalp video EEG (VEEG) and MEG in ABPE patients. *Results:* Eighteen ABPE patients were identified (nine girls and nine boys). Seizure onset ranged from 1.3 to 8.8 years (median, 2.9 years). Initial seizures consisted of focal motor seizures (15 patients) and absences/atypical absences (3). Seventeen patients had multiple seizure types including drop attacks (16), focal motor seizures (16), ENM (14), absences/atypical absences (11) and focal myoclonic seizures (10). VEEG showed centro-temporal spikes and CSWS in all patients. Magnetic resonance imaging (MRI) was reported as normal in all patients. MEGSSs were localized over the following regions: both Rolandic and sylvian (8), peri-sylvian (5), peri-Rolandic (4), parieto-occipital (1), bilateral (10) and unilateral (8). All patients were on more than two antiepileptic medications. ENM and absences/atypical absences were controlled in 14 patients treated with adjunctive ethosuximide. *Conclusion:* MEG localized the source of centro-temporal spikes and CSWS in the Rolandic-sylvian regions. Centro-temporal spikes, Rolandic-sylvian spike sources and focal motor seizures are evidence that ABPE presents with Rolandic-sylvian onset seizures. ABPE is therefore a unique, age-related and localization-related epilepsy with a Rolandic-sylvian epileptic focus plus possible thalamo-cortical epileptic networks in the developing brain of children.

Keywords: Epileptic negative myoclonus; Focal seizure; Atypical absence; Centro-temporal spike; Continuous spike and waves during sleep; Secondary bilateral synchrony

* Corresponding author. Address: Division of Neurology, The Hospital for Sick Children, 555 University Avenue, University of Toronto, Toronto, ON, Canada M5G 1X8. Tel.: +1 416 813 6660; fax: +1 416 813 6334.
E-mail addresses: hiotsubo@rogers.com, hiroshi.otsubo@sickkids.ca (H. Otsubo).

0387-7604/$ - see front matter © 2013 Published by Elsevier B.V. on behalf of The Japanese Society of Child Neurology.
http://dx.doi.org/10.1016/j.braindev.2012.12.011

Please cite this article in press as: Shiraishi H et al. Magnetoencephalography localizing spike sources of atypical benign partial epilepsy. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2012.12.011