Table 2 Comparison of patients with poor and good outcomes

	All patients (n = 21)	Poor outcome (n = 6)	95% CI	Good outcome (n = 15)	95% CI	p Value
Age, y	20.3 ± 17.9	27.5 ± 25.1	1.1-53.9	17.5 ± 14.2	9.6-25.3	NS
Sex, M	6 (28.6)	3 (50.0)	11.8-88.2	3 (20.0)	4.3-48.1	NS
Ingestion to enteritis, d	3.0 ± 0.8	2.8 ± 0.8	2.0-3.6	3.1 ± 0.9	2.6-3.6	NS
Enteritis to HUS, d	3.7 ± 1.5	3.2 ± 0.8	2.4-4.0	3.9 ± 1.7	2.9-4.8	NS
HUS to encephalopathy, d	1.8 ± 1.9	0.8 ± 0.8	0-1.6	2.2 ± 2.1	1.0-3.4	<0.05
Therapies						
HD	17 (81.0)	4 (66.7)	22.3-95.7	13 (86.7)	59.5-98.3	NS
PE	12 (57.1)	4 (66.7)	22.3-95.7	8 (53.3)	26.6-78.7	NS
PMX	7 (33.3)	1 (16.7)	0.4-64.1	6 (40.0)	16.3-67.7	NS
TM	10 (46.7)	1 (16.7)	0.4-64.1	9 (60.0)	32.3-83.7	NS
mPSL	12 (57.1)	1 (16.7)	0.4-64.1	11 (73.3)	44.9-92.2	<0.05
IVIg	13 (61.9)	3 (50.0)	11.8-88.2	10 (66.7)	44.9-92.2	NS
Laboratory values						
WBC, ×1,000/μL	39.8 ± 18.7	41.0 ± 11.5	29.0-53.2	39.2 ± 21.2	27.5-51.0	NS
Hb, g/dL	6.6 ± 1.7	7.5 ± 2.7	4.6-10.3	6.2 ± 1.0	5.7-6.7	NS
PLT, ×10,000/μL	1.9 ± 1.4	2.0 ± 0.8	1.2-2.8	1.9 ± 1.7	1.0-2.8	NS
AST, IU/L	144 ± 86	215 ± 117	93.2-338.4	116 ± 53	86.8-145.1	NS
Creatinine, mg/dL	4.4 ± 2.9	7.9 ± 2.8	4.9-10.9	3.0 ± 1.5	2.1-3.8	<0.01
CRP, mg/dL	14.8 ± 9.6	16.1 ± 9.1	6.6-25.7	14.2 ± 9.9	8.7-19.7	NS
Basal ganglia lesion	10 (47.6)	5 (83.3)	35.9-99.6	5 (33.3)	11.8-61.6	NS
Thalamus lesion	12 (57.1)	5 (83.3)	35.9-99.6	7 (46.7)	21.3-73.4	NS

Abbreviations: AST = aspartate aminotransferase; CI = confidence interval; CRP = C-reactive protein; Hb = hemoglobin; HD = hemodialysis; HUS = hemolytic-uremic syndrome; IVIg = IV immunoglobulin; mPSL = methylprednisolone; NS = not significant; PE = plasma exchange; PLT = platelets; PMX = polymyxin-B immobilized column direct hemoperfusion; TM = thrombomodulin; WBC = white blood cells.
Data are presented as mean ± SD or n (%).

DISCUSSION The most important findings in this case series are as follows: 1) we observed a high incidence of encephalopathy in STEC O111-HUS patients (21/34), especially in children (10/11), progressively leading to death in 5 patients, but to complete recovery in almost all surviving patients; 2) mPSL pulse therapy was potentially effective for encephalopathy; 3) neuroimaging showed marked cerebral edema in patients who died; and 4) more severe renal dysfunction led to a more severe neurologic outcome.

The most prevalent STEC serotype, STEC O157 infection, caused HUS in 6.3% and death in 0.6% (4.6% of STEC O157-HUS cases) of reported cases.[11] According to a recent large study in France, the frequency of neurologic involvement in patients with STEC-HUS is approximately 3%, and neurologic complications lead to death in 17% of those affected.[3] The STEC O104 outbreak in northern Germany in 2011 was characterized by a large number of patients with HUS (22%) and neurologic symptoms (26% of children, and 48% of adult patients with HUS)[6,7,12,13]; however, the mortality rates were 4.2% for patients with STEC O104-HUS, and 1.4% for all patients,[7] similar to those reported for STEC O157. The STEC O111 outbreak in Oklahoma in 2008 was also characterized by high frequencies of HUS (16.7%) and neurologic symptoms (46% of patients with HUS) but relatively low fatality rates, specifically 3.8% for patients with STEC O104-HUS, and 0.6% for all patients.[5] Compared with these data, the STEC O111 outbreak in Toyama was characterized by a high frequency of HUS (40%), encephalopathy in 62% for STEC O111-HUS, and death in 24%, 15%, and 5.8% for encephalopathy, HUS, and all patients, respectively.

We postulate 3 possible pathologic mechanisms for neurologic complications in STEC infection: direct Stx injury, inflammatory CNS responses, and neurotoxicity due to uremia. Stxs bind to globotriaosylceramide (Gb3), the receptor expressed on the surface of endothelial cells and neurons,[14] are internalized through receptor-mediated endocytosis, and inhibit protein synthesis through interaction with the 60S ribosomal subunit thereby inducing apoptotic cell death.[15,16] Indicative of endothelial cell injury, the

Table 3 Characteristics of patients with and without mPSL

Variable	mPSL (n = 12)	No mPSL (n = 9)	p Value
Age, y	14.3 ± 8.0	28.3 ± 24.3	NS
Sex, M	2 (16.7)	4 (44.4)	NS
Ingestion to enteritis, d	3.0 ± 1.0	3.0 ± 0.7	NS
Enteritis to HUS, d	3.8 ± 1.9	3.4 ± 0.7	NS
HUS to encephalopathy, d	2.3 ± 2.2	1.2 ± 1.2	NS
WBC, ×1,000/μL	40.0 ± 23.7	39.4 ± 9.9	NS
Hb, g/dL	6.2 ± 1.0	7.0 ± 2.3	NS
PLT, ×10,000/μL	1.7 ± 0.7	2.2 ± 2.1	NS
AST, IU/L	118.2 ± 53.0	179.6 ± 111.6	NS
Creatinine, mg/dL	3.4 ± 2.3	5.8 ± 3.3	NS
CRP, mg/dL	15.4 ± 11.2	14.0 ± 7.3	NS
Basal ganglia lesion	5 (41.7)	5 (55.6)	NS
Thalamus lesion	8 (66.7)	4 (44.4)	NS

Abbreviations: AST = aspartate aminotransferase; CRP = C-reactive protein; Hb = hemoglobin; HUS = hemolytic-uremic syndrome; mPSL = methylprednisolone; NS = not significant; PLT = platelets; WBC = white blood cells.
Data are presented as mean ± SD or n (%).

neuropathology in animal models injected with Stx2 shows lesions suggestive of ischemic damage and arteriolar necrosis due to thrombotic microangiopathy.[17,18] Stxs that injure endothelial cells may negatively affect the blood-brain barrier, and thereby infiltrating brain parenchyma,[14,19] where they can directly injure neurons and result in neuronal dysfunction.[20]

Proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) markedly increase the Gb3 content and Stx-binding to brain endothelial cells, resulting in increased cytotoxicity and upregulation of apoptotic cell death.[21–23] A rabbit model study in which animals were given IV Stx2 injections showed that in addition to neuronal apoptotic death, microglial activation and significant upregulation of TNF-α and IL-1β transcription occurs in the brain parenchyma.[18] Activated microglia are known to produce proinflammatory cytokines,[24] and TNF-α directly induces neurodegeneration through multiple pathways.[25–27] Proinflammatory cytokines are, therefore, closely related to the pathogenesis of STEC-encephalopathy. Gb3 is upregulated by proinflammatory cytokines, and these cytokines are, in turn, released through the interaction of Stxs with activated microglia.

The high fatality rate in the STEC O111 outbreak in Toyama resulted from progressive encephalopathy. MRI or CT of 4 patients who later died revealed acutely progressive cerebral edema and possible herniation on days 1 to 3 within 48 hours after previous imaging with no or little cerebral edema. These findings were confirmed by postmortem neuropathologic examination, which revealed severe noninflammatory cerebral edema and herniation in 3 patients so examined (patients 3, 6, and 8).[28] Previous reports of MRI findings in patients with neurologic complications associated with other STEC outbreaks, including the STEC O104 outbreak in Germany, did not describe acute and diffuse cerebral edema.[3,4,6,7] In addition, neither cerebral edema nor herniation was documented on postmortem examination in 5 fatal cases of STEC O104 in Germany.[7] Therefore, it is reasonable to consider that progressive encephalopathy leading to severe cerebral edema is characteristic of the STEC O111 infection in Toyama.

Clinical and neuroradiologic features and neuropathologic findings of diffuse noninflammatory cerebral edema are similar to those observed in Japanese children with infectious encephalopathy, especially cases associated with influenza.[29,30] Children with STEC O111-HUS developed encephalopathy (10/11) more frequently than adults (11/25), which has also been the case with influenza encephalopathy in Japan. During the acute stage of influenza encephalopathy, serum and CSF concentrations of inflammatory cytokines (i.e., TNF-α and IL-6) are abnormally high in many patients,[31,32] suggesting that cytokine storm has a major role in the pathogenesis. Vascular injury leading to brain edema has actually been ascribed to endothelial damage caused by cytokines.[29]

Corticosteroids suppress proinflammatory cytokine gene expression, and activate genes encoding inhibitors of inflammation.[33] mPSL, IVIg, and other therapies that suppress inflammatory cytokines have, therefore, been recommended for influenza encephalopathy.[9] mPSL therapy is effective for influenza encephalopathy caused by hypercytokinemia such as acute necrotizing encephalopathy, and improves neurologic outcomes.[9,34] Physicians in Toyama decided to treat patients with STEC O111-encephalopathy with mPSL and IVIg after May 1, 2011, based on clinical, radiologic, and pathologic similarity to influenza encephalopathy. We successfully showed that mPSL pulse therapy increased the probability of a good outcome. Indeed, no patient with STEC O111-encephalopathy died after mPSL therapy. Cytokine studies on affected patients in the STEC O111 outbreak in Toyama showed more severe hypercytokinemia in 11 patients with severe STEC O111-HUS (including 8 patients with encephalopathy) than in 3 with mild HUS without encephalopathy,[8] supporting the hypothesis that cytokine storm is important in the pathogenesis of STEC O111-encephalopathy. Although no specific therapy has been established for STEC-encephalopathy, plasma exchange, eculizumab, and immunoabsorption treatments have been proposed.[6] Corticosteroid therapy, especially mPSL pulse therapy, should be considered for the treatment of STEC-encephalopathy.

Progressive encephalopathy leading to severe cerebral edema and death is not observed in countries other than Japan. This may be because Japanese people are genetically more susceptible to infectious encephalopathy than people of other countries. Viral encephalopathy, most often secondary to influenza and human herpes virus 6, is the most prevalent type of encephalopathy in Japanese children.[29] Several syndromes, such as acute encephalopathy with biphasic seizures and late reduced diffusion, and acute necrotizing encephalopathy,[29,30,35] are by far more common in East Asia than in the rest of the world. The mechanisms underlying racial or regional differences are not fully understood; however, single nucleotide polymorphisms of several genes, such as those for the carnitine palmitoyltransferase II and adenosine A2a receptors, are reported to be risk factors for acute encephalopathy with biphasic seizures and late reduced diffusion.[36,37] Differences in such single nucleotide polymorphism frequencies between Japanese and other individuals may account for racial differences in neurologic symptoms associated with viral or STEC infections. It is also possible that the STEC O111 prevalent in Toyama was more toxic than the previous STEC, but bacteriologic studies to date have not elucidated the mechanism by which this specific strain caused many cases with severe complications.[38]

Renal function during the course of infection in patients with a poor outcome was worse than in individuals with a good outcome. Because uremia per se can cause brain dysfunction, and neurologic symptoms occur at the peak of renal dysfunction,[7] it is possible that more severe uremia caused severe neurologic symptoms resulting in accompanying poor outcomes. Neither hemodialysis nor plasma exchange affected the neurologic symptoms or outcome, which were compatible with a previous study.[6] In addition, some patients with STEC infection showed neurologic symptoms in the absence of renal dysfunction,[7,39] and 9% to 15% of patients with STEC-encephalopathy showed cerebral dysfunction before the onset of HUS.[40] These findings suggest that mechanisms other than uremia, such as the direct effects of Stxs and inflammatory responses in the CNS, may have major roles in the pathogenesis of STEC-encephalopathy.

Symmetrical lesions that we observed in our patients with STEC O111-encephalopathy in the lateral thalamus, basal ganglia, external capsule, and dorsal brainstem or cerebellum are similar to those reported previously in patients with STEC-encephalopathy.[3,4,6,7] This characteristic distribution may provide a radiologic clue for early diagnosis because, although it takes time for microbiologic identification of STEC, STEC-encephalopathy can be observed on the same day as HUS. Early diagnosis by radiologic identification of STEC-encephalopathy could be a useful tool promoting prevention of encephalopathy progression through use of the suggested treatments described herein.

Of interest, the ADC value revealed different patterns in the thalamus with reduced diffusion compared with the putamen and external capsule with increased diffusion in the acute stage of STEC O111-encephalopathy, suggesting that the former reflects cytotoxic edema, and the latter vasogenic edema, probably due to breakdown of the blood-brain barrier. Neuropathologic examination of 3 patients (patients 3, 6, and 8) revealed severe edema without inflammatory cells in both the thalamus and basal ganglia,[28] which could not explain the ADC difference. A neuropathologic study involving patients with STEC O104-encephalopathy revealed that astrogliosis and microgliosis were prominent in the thalamus and pons,[7] which were compatible with prominent cytotoxic edema in these regions. We know that Gb3 is highly expressed in neurons of all brain regions in patients with STEC O104 infection,[7] suggesting no correlation between Gb3 distribution and MRI lesions. We remain uncertain as to what determined the topographical pathology distribution seen on MRI.

Because we had to treat severely ill patients immediately without any evidence-based protocol at the beginning of this outbreak, the timing or combination of therapies for encephalopathy was not uniform. We did not perform multivariate statistics to confirm the effectiveness of mPSL because of the small number of patients. Definite treatment recommendations cannot, therefore, be drawn directly from the study.

AUTHOR CONTRIBUTIONS

J. Takanashi contributed to the design and conceptualization of the study, data collection, data analysis, data interpretation, statistical analysis, writing, literature search, and figures. H. Taneichi, T. Misaki, and Y. Yahata contributed to the data collection, data analysis, data interpretation, and manuscript revision. A. Okumura contributed to the design of the study, data analysis, data interpretation, and manuscript revision. Y. Ishida and T. Miyawaki contributed to the data collection and manuscript revision. N. Okabe and T. Sata contributed to the data collection, data analysis, data interpretation, and manuscript revision. M. Mizuguchi contributed to the design and conceptualization of the study, data collection, data analysis, data interpretation, and writing.

ACKNOWLEDGMENT

The authors thank Dr. Kazumasa Ogura, Department of Pediatrics, Fukui Red Cross Hospital, Fukui; Drs. Keiko Takada, Katsuhisa Inamura, and Toshiyuki Okamura, Department of Gastroenterology, Tonami General Hospital, Tonami; Dr. Michio Konishi, Department of Pediatrics, Tonami General Hospital, Tonami; Dr. Masaru Nakagawa, Department of Nephrology, Kanazawa Medical University, Kahoku-gun; Dr. Hisashi Kaneda, Department of Pediatrics, Toyama City Hospital, Toyama; Dr. Hideki Mizuno, Department of Medicine, Toyama City Hospital, Toyama; Dr. Satoshi Hiraide, Department of Nephrology and Hypertension, Seirei Yokohama Hospital, Yokohama; Dr. Teiichi Terasaki, Department of Medicine, Saiseikai Takaoka Hospital, Takaoka; Dr. Kazuya Inoki, Department of Gastroenterology, Yodogawa Christian Hospital,

Osaka; Dr. Shigeru Azuma, Department of Medicine, Koseiren Takaoka Hospital, Takaoka; Dr. Mondo Kuroda, Department of Pediatrics, Kanazawa University, Kanazawa; Dr. Kiyoki Kitagawa, Department of Nephrology, Kanazawa University, Kanazawa; Dr. Takata Hiroyuki, Toyama Red Cross Hospital, Toyama; Dr. Hiroki Misawa, Department of Medicine, Takaoka City Hospital, Takaoka; Dr. Mika Ito, Department of Obstetrics and Gynecology, University of Toyama; and Dr. Tomomi Tanaka, Department of Pediatrics, University of Toyama, Japan, for the clinical support and for collecting the data for this study.

STUDY FUNDING

No targeted funding reported.

DISCLOSURE

J. Takanashi was funded by a Grant-in-Aid for Research on Measures for Intractable Diseases (H25-Nanji-Ippan-009) from the Ministry of Health, Labor and Welfare, Japan; and a Grant-in-Aid for Scientific Research (B24390258) from Japan Society for the Promotion of Science; and is an editorial board member of *Brain and Development*. H. Taneichi was funded by a Grant for Research on Emerging and Re-emerging Diseases (H24-Shinko-Ippan-012) from the Ministry of Health, Labor and Welfare, Japan. T. Misaki and Y. Yahata report no disclosures. A. Okumura was funded by a Grant-in-Aid for Research on Measures for Intractable Diseases (H25-Nanji-Ippan-009) from the Ministry of Health, Labor and Welfare, Japan. Y. Ishida reports no disclosures. T. Miyawaki was funded by a Health Labour Sciences Research Grant (H23-TOKUBETU-SHITEI-004) from the Ministry of Health, Labor and Welfare, Japan. N. Okabe was funded by a Health Labour Sciences Research Grant (H23-TOKUBETU-SHITEI-004) from the Ministry of Health, Labor and Welfare, Japan. T. Sata was funded by a Health Labour Sciences Research Grant (H23-TOKUBETU-SHITEI-004) from the Ministry of Health, Labor and Welfare, Japan. M. Mizuguchi was funded by a Grant-in-Aid for Research on Measures for Intractable Diseases (H25-Nanji-Ippan-009) and a Grant for Research on Emerging and Re-emerging Diseases (H24-Shinko-Ippan-012), both from the Ministry of Health, Labor and Welfare, Japan; a Grant-in-Aid for Scientific Research (B24390258) from Japan Society for the Promotion of Science; and is an editor-in-chief of *Brain and Development*. Go to Neurology.org for full disclosures.

Received April 11, 2013. Accepted in final form November 12, 2013.

REFERENCES

1. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. Lancet 2005;365:1073–1086.
2. Pennington H. Escherichia coli O157. Lancet 2010;376: 1428–1435.
3. Nathanson S, Kwon T, Elmaleh M, et al. Acute neurological involvement in diarrhea-associated hemolytic uremic syndrome. Clin J Am Soc Nephrol 2010;5:1218–1228.
4. Donnerstag F, Ding X, Pape L, et al. Patterns in early diffusion-weighted MRI in children with haemolytic uremic syndrome and CNS involvement. Eur Radiol 2012; 22:506–513.
5. Piercefield EW, Bradley KK, Coffman RL, Mallonee SM. Hemolytic uremic syndrome after an Escherichia coli O111 outbreak. Arch Intern Med 2010;170:1656–1663.
6. Weissenborn K, Donnerstag F, Kielstein JT, et al. Neurologic manifestations of E coli infection-induced hemolytic-uremic syndrome in adults. Neurology 2012;79:1466–1473.
7. Magnus T, Röther J, Simova O, et al. The neurological syndrome in adults during the 2011 northern German E. coli serotype O104:H4 outbreak. Brain 2012;135:1850–1859.
8. Shimizu M, Kuroda M, Sakashita N, et al. Cytokine profiles of patients with enterohemorrhagic Escherichia coli O111-induced hemolytic-uremic syndrome. Cytokine 2012;60:694–700.
9. The Research Committee on the Clarification of the Etiology and on the Establishment of Therapeutic and Preventive Measures for Influenza Encephalopathy. *Guidelines for Influenza Encephalopathy*. Japan: Ministry of Health, Labor and Welfare; 2009:23–32.
10. Matano S, Inamura K, Konishi M, et al. Encephalopathy, disseminated intravascular coagulation, and hemolytic-uremic syndrome after infection with enterohemorrhagic Escherichia coli O111. J Infect Chemother 2012;18:558–564.
11. Gould LH, Demma L, Jones TF, et al. Hemolytic uremic syndrome and death in persons with Escherichia coli O157:H7 infection, Foodborne Diseases Active Surveillance Network sites, 2000–2006. Clin Infect Dis 2009; 49:1480–1485.
12. Loos S, Ahlenstiel T, Kranz B, et al. An outbreak of Shiga toxin-producing Escherichia coli O104:H4 hemolytic uremic syndrome in Germany: presentation and short-term outcome in children. Clin Infect Dis 2012; 55:753–759.
13. Frank C, Werber D, Cramer JP, et al. Epidemic profile of Shiga-toxin-producing Escherichia coli O104:H4 outbreak in Germany. N Eng J Med 2011;365:1171–1180.
14. Obata F, Tohyama K, Bonev AD, et al. Shiga toxin 2 affects the central nervous system through receptor globotriaosylceramide localized to neurons. J Infect Dis 2008; 198:1398–1406.
15. Jones NL, Islur A, Haq R, et al. Escherichia coli Shiga toxins induce apoptosis in epithelial cells that is regulated by the bcl-2 family. Am J Physiol Gastrointest Liver Physiol 2000;278:G811–G819.
16. Erwert RD, Eiting KT, Tupper JC, Winn RK, Harlan JM, Bannerman DD. Shiga toxin induces decreased expression of the antiapoptotic protein Mcl-1 concomitant with the onset of endothelial apoptosis. Microb Pathog 2003;35:87–93.
17. Fujii J, Kinoshita Y, Kita T, et al. Magnetic resonance imaging and histopathological study of brain lesions in rabbits given intravenous verotoxin 2. Infect Immun 1996;64: 5053–5060.
18. Takahashi K, Funata N, Ikuta F, Sato S. Neuronal apoptosis and inflammatory responses in the central nervous system of a rabbit treated with Shiga toxin-2. J Neuroinflammation 2008;5:11.
19. Goldstein J, Loidl CF, Creydt VP, Boccoli J, Ibarra C. Intracerebroventricular administration of Shiga toxin type 2 induces striatal neuronal death and glial alterations: an ultrastructural study. Brain Res 2007;1161:106–115.
20. Tironi-Farinati C, Loidl CF, Boccoli J, Parma Y, Fernandez-Miyakawa ME, Goldstein J. Intracerebroventricular Shiga toxin 2 increases the expression of its receptor globotriaosylceramide and causes dendritic abnormalities. J Neuroimmun 2010;222:48–61.
21. Ramegowda B, Samuel JE, Tesh VL. Interaction of Shiga toxins with human brain microvascular endothelial cells: cytokines as sensitizing agents. J Infect Dis 1999;180: 1205–1213.
22. Eisenhauer PB, Chaturvedi P, Fine RE, et al. Tumor necrosis factor alpha increases human cerebral endothelial cell Gb3 and sensitivity to Shiga toxin. Infect Immun 2001;69:1889–1894.
23. Stricklett PK, Hughes AK, Ergonul Z, Kohan DE. Molecular basis for up-regulation by inflammatory cytokines of Shiga toxin 1 cytotoxicity and globotriaosylceramide expression. J Infect Dis 2002;186:976–982.

24. Aloisi F. Immune function of microglia. Glia 2001;36: 165–179.
25. Yang L, Lindholm K, Konishi Y, Li R, Shen Y. Target depletion of distinct tumor necrosis factor receptor subtypes reveals hippocampal neuron death and survival through different signal transduction pathways. J Neurosci 2002;22: 3025–3032.
26. Akassoglou K, Bauer J, Kassiotis G, et al. Oligodendrocyte apoptosis and primary demyelination induced by local TNF/p55TNF receptor signaling in the central nervous system of transgenic mice: models for multiple sclerosis with primary oligodendrogliopathy. Am J Pathol 1998; 153:801–813.
27. Zhao X, Bausano B, Pike BR, et al. TNF-α stimulates caspase-3 activation and apoptotic cell death in primary septo-hippocampal cultures. J Neurosci Res 2001;64: 121–131.
28. Nishida N, Hata Y, Sasahara M, Ishii Y, Hamashima T, Shin J. Postmortem examination in patients with O111 infection [in Japanese]. Sata T, editor. Annual Report on Epidemiology, Microbiologic Features and Clinical Manifestations in EHEC/O111 Outbreak. Special Research (H23-TOKUBETU-SHITEI-004). Japan: Ministry of Health, Labor and Welfare of Japan; 2012:179–182.
29. Mizuguchi M, Yamanouchi H, Ichiyama T, Shiomi M. Acute encephalopathy associated with influenza and other viral infections. Acta Neurol Scand 2007;115:45–56.
30. Takanashi J. Two newly proposed encephalitis/encephalopathy syndromes. Brain Dev 2009;31:521–528.
31. Ichiyama T, Isumi H, Ozawa H, Matsubara T, Moroshima T, Furukawa S. Cerebrospinal fluid and serum levels of cytokines and soluble tumor necrosis factor receptor in influenza virus-associated encephalopathy. Scand J Infect Dis 2003;35:59–61.
32. Ichiyama T, Endo S, Kaneko M, Ishumi H, Matsubara T, Furukawa S. Serum cytokine concentrations of influenza-associated acute necrotizing encephalopathy. Pediatr Int 2003;45:734–736.
33. Flammer JR, Rogatsky I. Glucocorticoids in autoimmunity: unexpected targets and mechanisms. Mol Endocrinol 2011;25:1075–1086.
34. Okumura A, Mizuguchi M, Kidokoro H, et al. Outcome of acute necrotizing encephalopathy in relation to treatment with corticosteroids and gammaglobulin. Brain Dev 2009;31:221–227.
35. Takanashi J, Oba H, Barkovich AJ, et al. Diffusion MRI abnormalities after prolonged febrile seizures with encephalopathy. Neurology 2006;66:1304–1309.
36. Shinohara M, Saitoh M, Takanashi JI, et al. Carnitine palmitoyl transferase II polymorphism is associated with multiple syndromes of acute encephalopathy with various infectious diseases. Brain Dev 2011;33:512–517.
37. Shinohara M, Saitoh M, Nishizawa D, et al. ADOR2A polymorphism predisposes children to encephalopathy with febrile status epilepticus. Neurology 2013;80:1–6.
38. Watahiki M, Ohnishi M, Sekizuka T. Summary of microbiologic research (in Japanese). Sata T, editor. Annual Report on Epidemiology, Microbiologic Features and Clinical Manifestations in EHEC/O111 Outbreak. Special Research (H23-TOKUBETU-SHITEI-004). Japan: Ministry of Health, Labor and Welfare of Japan; 2012;99–103.
39. Siegler RL. Spectrum of extrarenal involvement in post-diarrheal hemolytic-uremic syndrome. J Pediatr 1994;125: 511–518.
40. Ahrens F, Ludwig K, Terstegge K, Querfeld U. Encephalopathy and exposure to Shiga toxin without evidence of haemolytic uraemic syndrome. Eur J Pediatr 2002;16: 462–463.

A Child with Three Episodes of Reversible Splenial Lesion

Takeshi Kouga[1] Mizue Iai[1] Sumimasa Yamashita[1] Noriko Aida[2] Jun-ichi Takanashi[3] Hitoshi Osaka[1]

[1] Division of Neurology, Kanagawa Children's Medical Center, Yokohama, Japan
[2] Division of Radiology, Kanagawa Children's Medical Center, Yokohama, Japan
[3] Department of Pediatrics, Kameda Medical Center, Kamogawa, Japan

Address for correspondence Hitoshi Osaka, MD, PhD, Division of Neurology, Kanagawa Children's Medical Center, 2-138-4 Mutsukawa, Minami-ku, Yokohama-shi, Kanagawa 232-8555, Japan (e-mail: hosaka@kcmc.jp).

Neuropediatrics 2013;44:199–202.

Abstract

In this study, we report the case of an 8-year-old girl who had three episodes of reversible splenial lesion of the corpus callosum (SCC) in 2 years. Vomiting, hypoglycemia, and fever were followed by altered consciousness and diminished muscle tone. In each episode, the clinical manifestations and abnormalities detected during magnetic resonance imaging resolved in 2 weeks. Transient alteration of vision and spike discharges revealed by interictal electroencephalogram implied the SCC lesions were related to epileptic activities. At follow-up, the patient had not presented with SCC lesions or altered consciousness for more than 4 years after undergoing carbamazepine treatment. Our case is the first report of a patient who presented with three episodes of reversible splenial lesion.

Keywords
- encephalitis
- encephalopathy
- corpus callosum
- splenium
- reversible
- epilepsy

Introduction

Magnetic resonance imaging (MRI) findings of a reversible lesion in the splenium of the corpus callosum (SCC) have been reported in patients with epilepsy receiving antiepileptic drugs, clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS),[1] ischemia, neurodegeneration, and autoimmune disease. In general, the clinical prognosis is good without subsequent complications. Although there are various theories for reversible splenial lesions, there is no clear explanation for the finding.[2,3] Here, we report a young girl who presented with an SCC lesion three times. She is free from the splenial lesion and clinical manifestations after beginning carbamazepine treatment, which suggests her condition was related to epileptic activities.

Case Report

An 8-year-old girl presented to us who had been born with no complications and had developed normally. At 1 year and 8 months, she developed generalized tonic seizures after diarrhea, without abnormalities on head computed tomography and electroencephalography (EEG). Her paternal cousin had shown febrile seizures. Subsequently, our patient showed three episodes of reversible splenial lesion as described below.

First Episode (at Age 2 Years, 2 Months)

Three days after fever at 39°C, lack of eye contact indicated tonic seizure with upper limbs in flexion for about 1 minute at 30 minutes after vomiting. The tonic seizure was controlled by diazepam and chloral hydrate administrated in the emergency room. Ambiguous speech, lack of eye contact, and diminished muscle tone were observed the next day. No enhancements on T1-weighted images (►**Fig. 1A**) were observed, but T2-weighted images on MRI showed hyperintense SCC lesions (►**Fig. 1B**).

She was admitted and treated with fluid replacement, glycerin, and phenobarbital to prevent recurrent convulsions. Although the symptoms were improved by the next day of hospitalization, her consciousness worsened on the 3rd hospital day. No additional therapy was begun because computed tomography findings were normal. On the 10th hospital day, she was able to maintain an upright position. She was

received
May 6, 2012
accepted after revision
October 8, 2012
published online
December 19, 2012

DOI http://dx.doi.org/10.1055/s-0032-1330854.
ISSN 0174-304X.

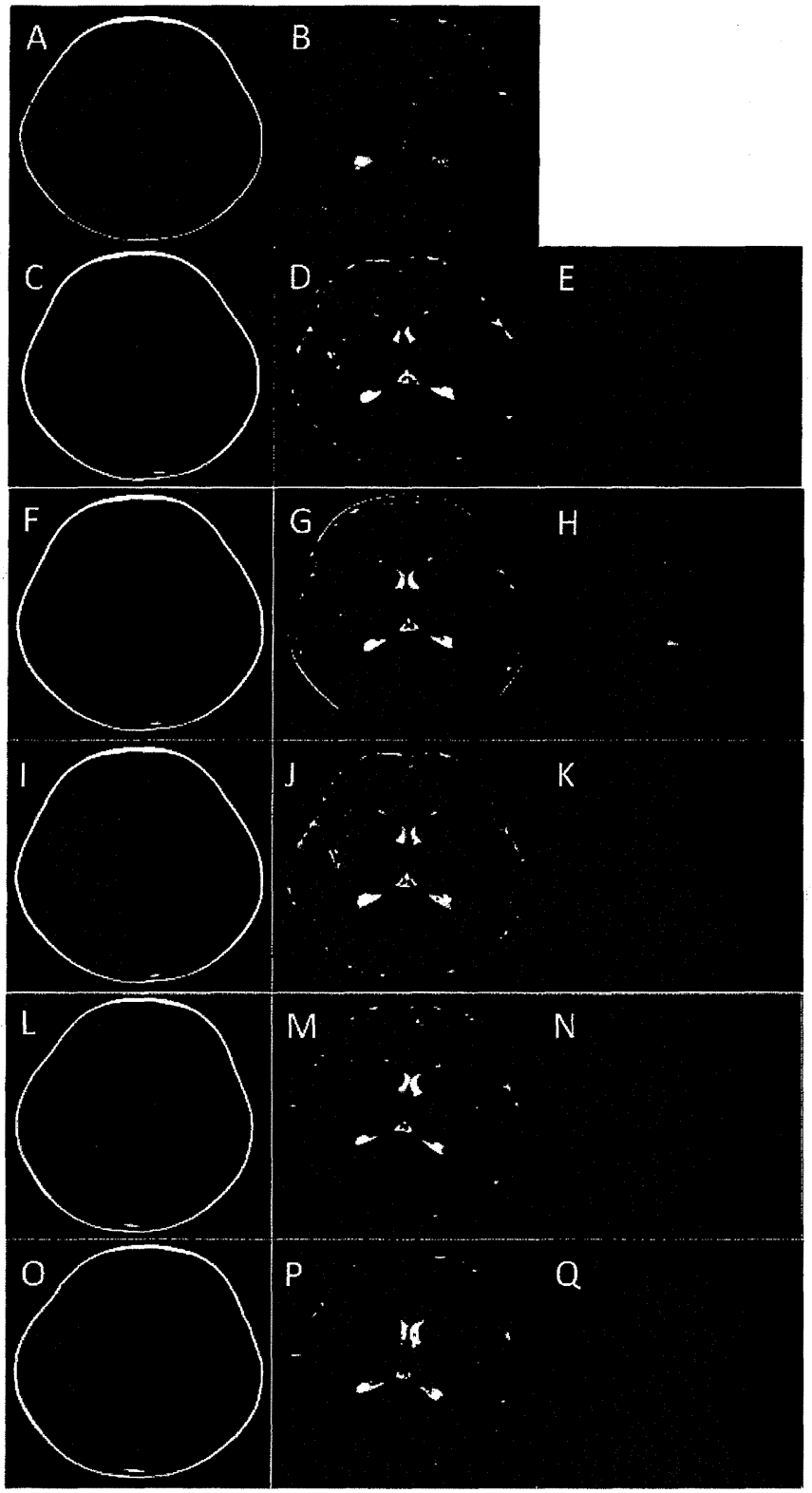

Fig. 1 Magnetic resonance images of the first episode (A, B: on admission; C–E: 2 months after the admission), the second episode (F–H: on admission; I–K: on the 8th hospital day), and the third episode (L–N: on admission; O–Q: on the 6th hospital day). Diffusion-weighted image in the first episode on admission was not performed. Hyperintense signal in the splenial lesion of the corpus callosum (SCC) on T2-weighted image in the first episode (B), on T2-weighted and diffusion-weighted images in the second episode (G, H), and in the third episode (M, N) completely disappeared at 2 months after (D), 7 days after (J, K), and 5 days after (P, Q). Except for SCC lesions on diffuse-weighted and T2-weighted images, no other abnormal enhancements were observed, including on T1-weighted images.

discharged on the 11th hospital day. The SCC lesion was resolved as indicated by MRI taken 2 months after discharge (►**Fig. 1C–E**).

Second Episode (at Age 2 Years, 6 Months)

The patient was received for sutures and antibiotics after sustaining mouth trauma by falling. Two days later rolling of the eyes and diminished muscle tone occurred after vomiting. She had difficulties in maintaining an upright position and walking. No abnormal enhancements were observed on T1-weighted images (►**Fig. 1F**), but T2-weighted and diffuse-weighted images on MRI showed hyperintense SCC lesions (►**Fig. 1G, H**).

She was admitted and treated with fluid replacement and glycerin. After hospitalization inconsistent speech and crying continued for a few days. These symptoms improved gradually, and the patient was recovered fully on the 5th hospital day. MRI on the 8th hospital day showed no abnormal findings (►**Fig. 1I–K**). She was discharged on the 9th hospital day.

Third Episode (at Age 3 Years, 4 Months)

Deviating eyes and generalized tonic convulsion for a few seconds developed after diarrhea, vomiting, and fever at approximately 39°C. In addition, she showed difficulty in maintaining a sitting or standing position with diminished muscle tone. No abnormal enhancements were observed on T1-weighted images (►**Fig. 1L**), but T2-weighted and diffuse-weighted images on MRI showed hyperintense SCC lesions (►**Fig. 1M, N**).

She was treated with fluid replacement and glycerin. On admission day she let out strange noises and could not understand simple verbal instructions. On the 2nd hospital day she was able to speak almost normally, and on the 6th hospital day she walked normally. MRI on the 6th hospital day showed no abnormal findings (►**Fig. 1O–Q**). She was discharged on the 7th hospital day.

Throughout these three episodes her biochemical analyses were normal except for hypoglycemia (45 to 58 mg/dL), increased blood ketone (609 to 870 µmol/L for acetoacetic acid, 4,590 to 5,417 µmol/L for 3-hydroxybutyric acid), mild metabolic acidosis (pH: 7.29 to 7.34), and decreased sodium (133 mEq/L, only in the third episode). Cerebrospinal fluid (CSF) examinations (only in the first episode) revealed normal cell counts and protein and glucose levels. Urine analyses were also normal. CSF polymerase chain reaction, cultures for blood in stool, and intravenous-contrast MRI were not performed.

After the third episode the patient complained of transient alteration of vision as "I can't see Mam," and the EEG at age 3 years, 9 months showed spike discharges in the occipital region during sleep (►**Fig. 2A**). She began to be treated with carbamazepine treatment at age 4 years because some epileptic activities were related to her episodes. The spike

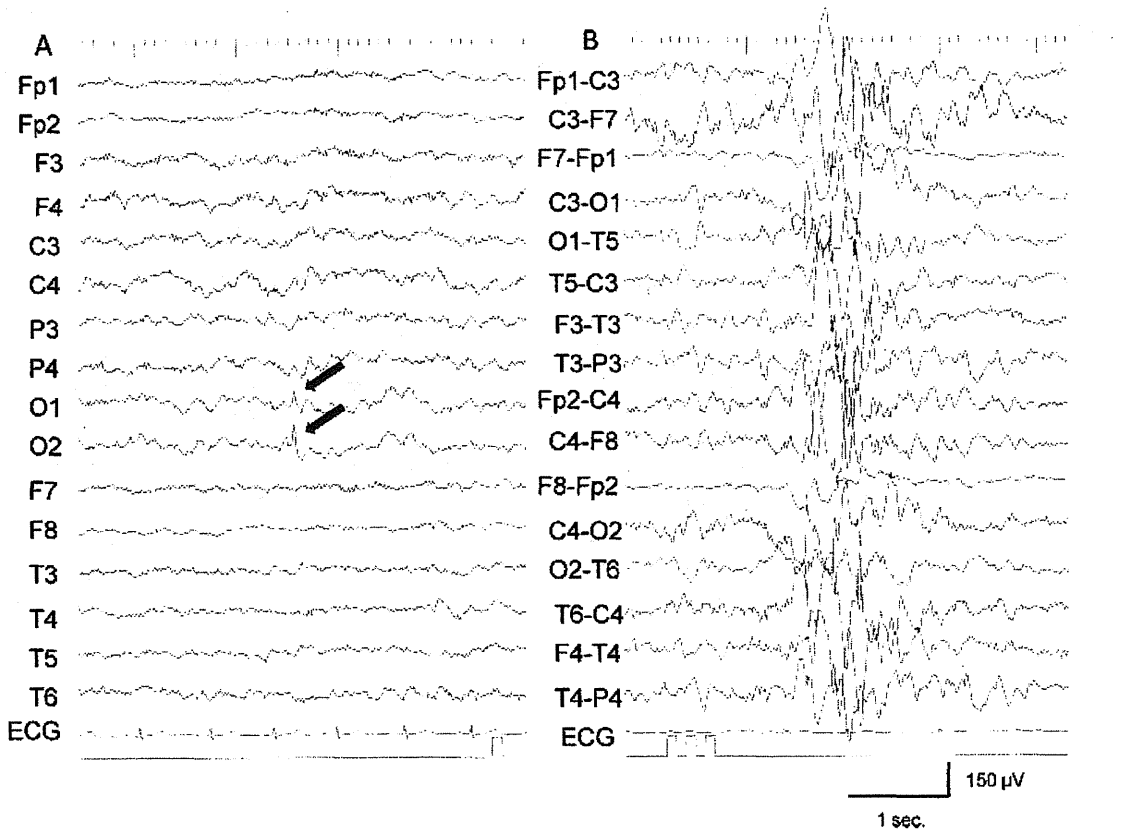

Fig. 2 Electroencephalography during sleep showed spike discharges in the occipital region (arrows) at age 3 years, 9 months (A) and diffuse spike and wave complexes at age 5 years, 5 months (B). The latter observation was reproduced at a later age.

discharges in the occipital region were not observed after carbamazepine treatment. EEG at age 5 years, 5 months showed diffuse spike and wave complexes during sleep (►**Fig. 2B**), which were reproducible in the EEG at age 7 years, 4 months. Currently, she is free from similar clinical episodes for more than 4 years.

Discussion

Various causes of reversible splenial lesion have been reported, including viral infection, hemolytic-uremic syndrome caused by *Escherichia coli* O-157, febrile seizure, frequent convulsion, antiepileptic drugs (especially phenytoin and carbamazepine), drug allergy, anticancer drugs, and radiation.[2,4] In addition, a correlation between hyponatremia and SCC lesion has been reported.[4] Alterations of the arginine-vasopressin system have been proposed as a cause.[4–6] However, a plausible mechanism of pathogenesis for SCC lesion has not been identified.[2,3]In the differential diagnosis of acquired lesions in the SCC, acute disseminated encephalomyelitis, extrapontine myelinolysis, MERS, ischemia, neurodegeneration, and autoimmune disease should be considered.[2,7]

Our patient showed altered consciousness represented by abnormal behavior such as strange noises and inability to understand simple verbal instructions followed by prodromal symptoms such as fever, vomiting, and convulsion. She was not able to maintain a standing/sitting position due to diminished muscle tone. The benign short clinical course, reversible lesions in the SCC, and no specific findings from blood, CSF, and urine examinations suggested MERS. Acute disseminated encephalomyelitis and extrapontine myelinolysis are unlikely as the MRIs during the three episodes disclosed. Because all episodes were followed by vomiting and mild hypoglycemia, we cannot preclude the possibility that she has a metabolic disease or endocrine disease not identified by urine organic acid analysis, tandem mass analysis, and carnitine fraction analysis.

Although she showed similar symptoms to epileptic seizures in each episode (tonic seizure, rolling of the eyes, and deviating eyes and generalized tonic convulsion in the first, second, and third episode, respectively), we were unable to obtain a diagnosis of epilepsy during each episode due to the lack of definite interictal paroxysmal discharge and altered consciousness lasting more than a few days. The complaint of a transient alteration of vision and spikelike discharges in the occipital region on EEG after the third episode suggested her episodes were related to epileptic discharges. After the initiation of carbamazepine, she is currently free from altered consciousness and SCC lesions for more than 4 years. Although we did not reach a conclusive diagnosis underlying the SCC lesions, her clinical course suggests that epilepsy was associated with her episodes. Although there are reports that transient ischemia or cytotoxic edema induced by frequent convulsions may be related to SCC lesions,[1,5] a correlation between epilepsy or epileptic discharge and SCC lesion remains unresolved. However, searching for epilepsy-related transient splenial lesions if new convulsions or a change of the EEG findings appears may be useful.

Our case may be the first report of repeated SCC lesions in the English literature. Previously, a Japanese case was reported that demonstrated two episodes of benign convulsions with gastroenteritis with transient splenial lesions.[8] Our patient also experienced a seizure after diarrhea. Both diarrhea-associated benign infantile seizures and mild encephalitis with SCC lesions have been reported, mainly in Asian countries. Genetic factors may underline the pathogenesis of her repeated SCC lesion.

Conflict of Interest

The authors declare no potential conflict of interest.

References

1 Tada H, Takanashi J, Barkovich AJ, et al. Clinically mild encephalitis/encephalopathy with a reversible splenial lesion. Neurology 2004; 63(10):1854–1858
2 Yeh IB, Tan LCS, Sitoh YY. Reversible splenial lesion in clinically mild encephalitis. Singapore Med J 2005;46(12):726–730
3 Takanashi J, Imamura A, Hayakawa F, Terada H. Differences in the time course of splenial and white matter lesions in clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS). J Neurol Sci 2010;292(1–2):24–27
4 Gürtler S, Ebner A, Tuxhorn I, Ollech I, Pohlmann-Eden B, Woermann FG. Transient lesion in the splenium of the corpus callosum and antiepileptic drug withdrawal. Neurology 2005;65(7): 1032–1036
5 Mirsattari SM, Lee DH, Jones MW, Blume WT. Transient lesion in the splenium of the corpus callosum in an epileptic patient. Neurology 2003;60(11):1838–1841
6 Parikh NC, Kulkarni M. Transient and reversible focal lesion involving the splenium of the corpus callosum in a person with epilepsy. Ann Indian Acad Neurol 2008;11(2):123–124
7 Vollmann H, Hagemann G, Mentzel HJ, Witte OW, Redecker C. Isolated reversible splenial lesion in tick-borne encephalitis: a case report and literature review. Clin Neurol Neurosurg 2011;113(5): 430–433
8 Morioka S, Otabe O, Uehara H, et al. Recurrence of transient splenial lesions in a child with "benign convulsions with gastroenteritis". [in Japanese] No To Hattatsu 2010;42(6):449–453

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

Serum and CSF biomarkers in acute pediatric neurological disorders

Takashi Shiihara [a,*], Taeko Miyake [b], Sakiko Izumi [b], Susumu Sugihara [a], Mio Watanabe [a], Jun-ichi Takanashi [c], Masaya Kubota [d], Mitsuhiro Kato [e]

[a] *Department of Neurology, Gunma Children's Medical Center, 779 Shimohakoda, Hokkitsu-machi, Shibukawa, Gunma 377-8577, Japan*
[b] *Department of Laboratory Medicine, Gunma Children's Medical Center, 779 Shimohakoda, Hokkitsu-machi, Shibukawa, Gunma 377-8577, Japan*
[c] *Department of Pediatrics, Kameda Medical Center, 929 Higashi-cho, Kamogawa-shi, Chiba 296-8602, Japan*
[d] *Division of Neurology, National Center for Child Health and Development, 2-10-1 Ohkura, Setagaya-Ku, Tokyo 157-8535, Japan*
[e] *Department of Pediatrics, Yamagata University Faculty of Medicine, 2-2-2 Iida-Nishi, Yamagata-shi, Yamagata 990-9585, Japan*

Received 28 February 2013; received in revised form 19 April 2013; accepted 19 June 2013

Abstract

Background: There have been numerous reports regarding serum or cerebrospinal fluid (CSF) biomarkers in various disorders; however, the validities of such biomarkers for more precise diagnoses and prognosis estimates remain to be determined, especially in pediatric patients with neurological disorders. *Methods:* Serum/CSF S100B, neuron-specific enolase, and total tau (tTau) were measured in various acute pediatric neurological disorders, and their usefulness for diagnostic and prognostic predictions was validated using receiver operating characteristic curves and area under the curve (AUC) analysis. *Results:* A total of 336 serum and 200 CSF specimens from 313 patients were examined, and we identified statistically significant differences that were relevant from diagnostic and prognostic viewpoints. CSF and serum tTau levels could be good predictors for diagnosis (CSF tTau; AUC = 0.76) and prognosis (serum tTau; AUC = 0.78). *Conclusions:* Both CSF and serum tTau levels could be useful for precise diagnostic and prognostic estimations in acute pediatric neurological disorders. Further studies are needed to clarify the clinical significance of such biomarkers.

Keywords: S100B; Neuron-specific enolase; Total tau; Receiver operating characteristic curves; Area under the curve

1. Introduction

There are many kinds of pediatric neurological disorders with acute symptoms, such as headache, altered consciousness, seizures, paralysis, and ataxia [1]. Pediatricians or pediatric neurologists who treat these patients use various approaches, such as history taking, physical/neurological examinations, routine laboratory tests, conventional cerebrospinal fluid (CSF) examinations, electroencephalography, and brain imaging to identify the underlying cause. Due to limited time and resources, it would be useful to develop more precise diagnostic and prognostic predictions, which are sometimes difficult to attain, especially in the early stages of these disorders. For more than a decade, there have been reports about serum/CSF biomarkers that are useful in identifying various neurological disorders, at least in study settings [2–9]. We examined serum and CSF S100B, neuron-specific enolase (NSE), and total tau (tTau), which are glial, neuronal, and axonal damage markers, respectively, in patients with acute encephalopathy with biphasic seizures and late reduced diffusion. We found that all 3 biomarker levels were significantly increased and useful for diagnosis [3]. We hope to evaluate the usefulness of these markers as diagnostic and prognostic predictors in other diseases.

* Corresponding author. Tel.: +81 279 52 3551; fax: +81 279 52 2045.
E-mail address: shiihara-ind@umin.net (T. Shiihara).

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.2013.06.011

Please cite this article in press as: Shiihara T et al. Serum and CSF biomarkers in acute pediatric neurological disorders. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.06.011

2. Methods

2.1. Patients

From June 2007 to August 2012, patients were enrolled in the study mainly via mailing lists for Japanese pediatric neurologists or pediatricians, such as the Zao Seminar Mailing List (available at: https://sites.google.com/site/zaoseminar/) and the Japanese Pediatric Conferences Mailing List (available at: https://jpmlc.org/). Diagnoses were made by the attending physicians and later confirmed for the purpose of this study by examination of the available clinico–radiological information. We asked the attending physicians to provide each patient's clinical course and prognosis at the most recent visit. To evaluate the prognosis, the degree of disability was scored with the modified Rankin scale (mRS), which ranged from 1: no residual disability to 4: death (Table 1) [10]. Ethics approval was obtained from the Gunma Children's Medical Center institutional review board, and written informed consent was provided by the patients' parents.

2.2. Biomarker assays

Serum and CSF samples were obtained from each patient at any point during the disease and immediately stored at −80 °C until they were analyzed. Commercially available sandwich enzyme-linked immunosorbent assays (ELISA) for human S100B (BioVendor, Modrice, Czech Republic), NSE (Alpha Diagnostic International, Inc., San Antonio, Texas, USA), and tTau (Invitrogen Corp., Carlsbad, California, USA) were carried out according to the manufacturers' protocols [3]. The detectable range for each ELISA kit was 50–2000 pg/ml, 5–200 ng/ml, and 31.2–2000 pg/ml for S100B, NSE, and tTau protein, respectively.

2.3. Statistical analysis

Data were expressed as the median and interquartile range (IQR) unless otherwise specified. Statistical analysis was performed with the statistical package R (version 2.15.2, available as a free download from http://www.r-project.org). Comparisons were performed between numerical variables with the Wilcoxon rank-sum test and between proportions with the proportion test. For multiple comparisons between numerical variables or proportions, the Kruskal–Wallis rank sum test or the proportion test were performed, then, if there were significant differences, the pairwise Wilcoxon rank-sum tests or the pairwise proportion tests were performed, adjusted with Holm's method. A P-value < 0.05 was considered statistically significant. As a measure of binary decision performance, the receiver operating characteristic (ROC) curves were assessed, using the area under the curve (AUC) with Bootstrap method [11]. An optimal threshold value (cutoff point) was selected as the situation maximizing the Youden index (Youden index = sensitivity + specificity−1) [12].

3. Results

We collected 497 serum and 274 CSF specimens from 372 patients with various disorders. To evaluate the usefulness of serum/CSF biomarkers as diagnostic or prognostic predictors in the early phase of acute pediatric neurological disorders, we only used specimens taken within 5 days of illness (DOI; the first day of neurological symptoms was regarded as DOI 0) and only investigated diagnostic categories with specimens from more than 5 patients. Thus, 336 serum and 200 CSF specimens from 313 patients were available for evaluation (median age, 2 years; IQR, 1–5 years; male: female ratio, 160:153). Diagnostic categories were as follows (in alphabetical order); acute encephalitis/encephalopathy (AEE), aseptic meningitis (AM), afebrile seizures (AS), controls (CTR), febrile seizures (FS), and septic meningitis (SM). AEE comprised various types of acute encephalitis and encephalopathy, such as acute disseminated encephalomyelitis, acute encephalopathy with biphasic seizures and late reduced diffusion, and acute encephalitis with refractory repetitive partial seizures [13,14]. AS included epileptic seizures or gastroenteritis-related convulsions [15]. CTR included patients who were suspected to have a neurological disorder or involvement, but testing revealed that they did not, such as extra-cerebral infections, Kawasaki disease, and blood disorders. The study

Table 1
Modified Rankin scale.

Score	Description
1	No residual disability; the child attends regular education and does not need remedial teaching[a]
2	Mild residual disability; the child is able to attend regular education but needs remedial teaching because of mild motor disturbances, mild learning disability, or both
3	Severe residual disability; the child has a severe motor deficit (needs braces or wheelchair), severe learning disability, or both, attends a school for special education or is confined to a daily care centre
4	Death

[a] For patient who has underlying condition and disability, the score is determined as 1, unless the disability is worsened after the event.

Please cite this article in press as: Shiihara T et al. Serum and CSF biomarkers in acute pediatric neurological disorders. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.06.011

Table 2
Characteristics of study populations.

Diagnosis	Number	Age (year)	Gender (M:F)	Sampling time (DOI)	mRS
AEE	88	3 (1–6)	37:51	1 (0–3)	2 (1–3)
AM	15	4 (2.5–8.5)	10:5	0 (0–1)	1 (1–1)
AS	52	2 (0.75–4)	20:32	0 (0–0)	1 (1–1)
CTR	85	3.5 (1–8)	40:45	Not available	1 (1–1)
FS	51	1 (1–3)	40:11	0 (0 – 1)	1 (1–1)
SM	22	1 (0–2.5)	13:9	1 (0–2)	1 (1–1)

AEE, Acute encephalitis/encephalopathy; AM, aseptic meningitis; AS, Afebrile seizures; CTR, control; FS, Febrile seizures; SM, septic meningitis; DOI, day of illness, mRS, modified Rankin scale.
Numerical variables are expressed as median (inter quartile range). There were statistically significant differences in age (between AEE and SM*), mRS (between AEE and AM**, AS**, CTR**, FS**, SM*; SM and CTR*), Gender (between FS and AEE**, AS**, CTR**), and sampling time (between AEE and AS**, FS**; SM and AS**) (* denoting P-value less than 0.05, ** less than 0.01).

population characteristics were summarized, and we found statistically significant differences in age, mRS, gender, and sampling time between diagnostic groups (Table 2).

3.1. Serum and CSF biomarkers between each diagnostic group

Initially, the serum and CSF levels of S100B, NSE, and tTau were compared between each diagnostic group (Fig. 1). There were statistically significant differences in serum NSE between AEE (median, 14.5 ng/ml; IQR, 6.0–35.8) and AS (median, 5.0 ng/ml; IQR, 5.0–8.0; $P < 0.001$), AEE and CTR (median, 8.0 ng/ml; IQR, 5.0–13.1; $P = 0.011$), AEE and FS (median, 5.9 ng/ml; IQR, 5.0–11.2; $P = 0.001$), AS and CTR ($P = 0.030$), and AS and SM (median, 9.0 ng/ml; IQR, 5.5–18.0; $P = 0.017$). Significant difference were also observed from serum tTau: AEE (median, 31.2 pg/ml; IQR, 31.2–292.5) and AS (median, 31.2 pg/ml; IQR, 31.2–31.2; $P < 0.001$), AEE and CTR (median, 31.2 pg/ml; IQR, 31.2–31.2; $P < 0.001$), AEE and FS (median, 31.2 pg/ml; IQR 31.2–50.0; $P = 0.006$), AS and SM (median, 40.0 pg/ml; IQR, 31.2–122.5; $P = 0.009$), CTR and SM ($P < 0.001$). For CSF S100B, we observed significant differences between AEE (median, 90.0 pg/ml; IQR 58.7–300.0) and CTR (median, 50.0 pg/ml; IQR, 50.0–50.0; $P < 0.001$), AEE and FS (median, 51.5 pg/ml; IQR, 50.0–77.4; $P = 0.011$), CTR and SM (median, 130.0 pg/ml; IQR, 79.6–272.2; $P < 0.001$), and FS and SM ($P = 0.006$). For CSF tTau, we found significant differences between AEE (median, 230.0 pg/ml; IQR, 116.0–800.0) and FS (median, 100.0 pg/ml; IQR, 50.0–141.8; $P = 0.002$).

3.2. Serum and CSF biomarkers between patients with good and poor prognoses

Next, the serum and CSF levels of S100B, NSE, and tTau were compared between patients with good and poor prognoses (defined as mRS of 1–2 and 3–4, respectively) (Fig. 2). The levels of all the measured biomarkers were significantly higher in patients with poor prognosis than in those with good prognosis, i.e., serum S100B, good (median, 50.0 pg/ml; IQR 50.0–70.4) vs. poor (median, 66.6 pg/ml; IQR 50.0–581.0; $P < 0.001$); serum NSE, good (median, 7.5 ng/ml; IQR, 5.0–15.0) vs. poor (median, 16.3 ng/ml; IQR, 7.3–132.9; $P < 0.001$); serum tTau, good (median, 31.2 pg/ml; IQR 31.2–40.0) vs. poor (median, 227.7 pg/ml; IQR, 31.2–1603.0; $P < 0.001$); CSF S100B, good (median, 60.0 pg/ml; IQR, 50.0–120.0) vs. poor (median, 182.8 pg/ml; IQR, 81.7–340.0; $P < 0.001$); CSF NSE, good (median, 5.0 ng/ml; IQR 5.0–5.0) vs. poor (median, 5.0 ng/ml; IQR, 5.0–7.2; $P = 0.020$); and CSF tTau, good (median, 118.7 pg/ml; IQR, 50.0–294.2) vs. poor (median, 319.3 pg/ml; IQR, 120.8–1900.0; $P = 0.002$).

3.3. Evaluations of diagnostic and prognostic validities, using ROC curve analyses

Finally, in order to evaluate diagnostic and prognostic validities, we applied ROC curve analyses. To qualify the diagnostic and prognostic validities, we analyzed each biomarker's ability to distinguish AEE from FS and between poor and good prognoses. Then we drew the ROC curves and calculated each AUC (Fig. 3). AUCs for diagnosis were as follows: serum S100B, 0.58, 95% confidence interval (CI) 0.50–0.66; serum NSE, 0.71, 95% CI 0.62–0.79; serum tTau, 0.68, 95% CI 0.60–0.76; CSF S100B, 0.72, 95% CI 0.61–0.82; CSF NSE, 0.62, 95% CI 0.56–0.68; and CSF tTau, 0.76, 95% CI 0.64–0.86. AUCs for prognosis were as follows: serum S100B, 0.64, 95% CI 0.56–0.73; serum NSE, 0.71, 95% CI 0.60–0.81; serum tTau, 0.78, 95% CI 0.70–0.86; CSF S100B, 0.72, 95% CI 0.59–0.82; CSF NSE, 0.61, 95% CI 0.49–0.73; and CSF tTau, 0.72, 95% CI 0.60–0.83. Furthermore, optimal threshold values were calculated for biomarkers with AUC > 0.75. The values for CSF tTau (AUC = 0.76) to distinguish AEE from FS were 156.7 pg/ml (Youden index 0.59), sensitivity

Please cite this article in press as: Shiihara T et al. Serum and CSF biomarkers in acute pediatric neurological disorders. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.06.011

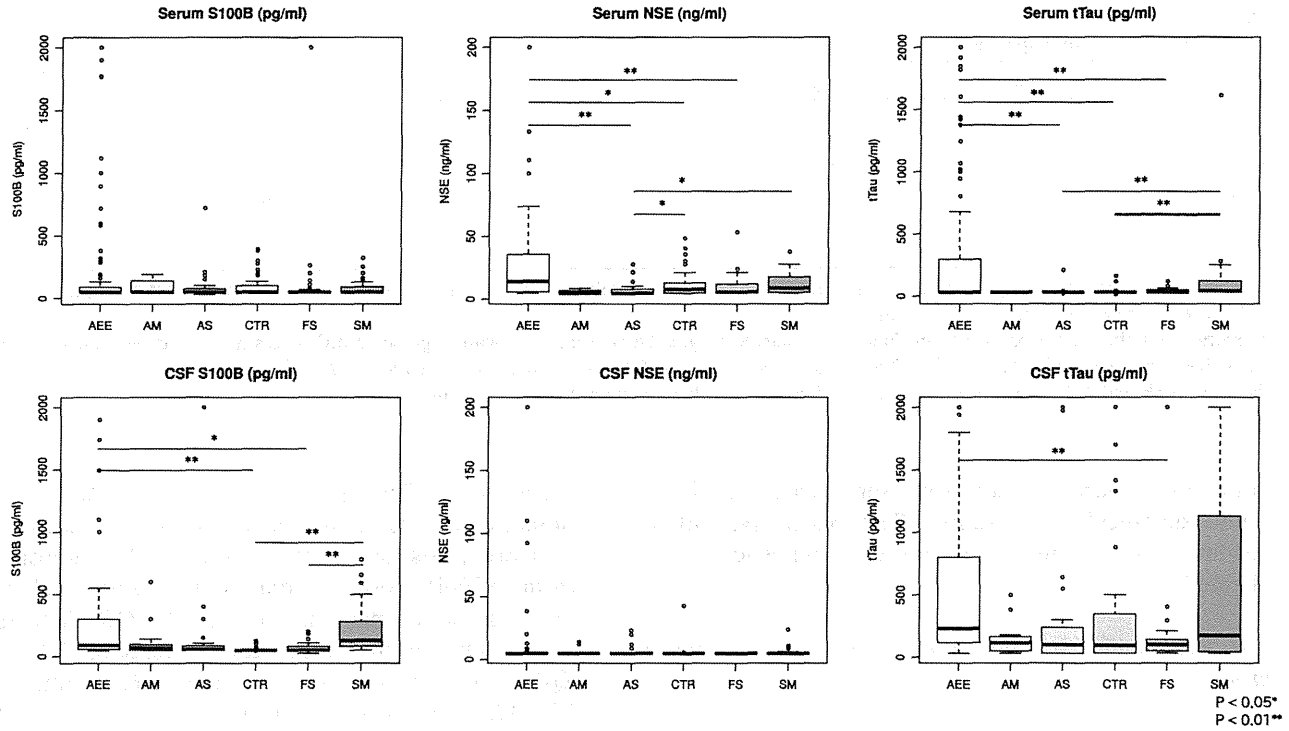

Fig. 1. Boxplot of serum (top) and CSF (bottom) levels of S100B (left), NSE (middle), and tTau (right) from patients with acute encephalitis/encephalopathy, AEE; aseptic meningitis, AM; afebrile seizures, AS; control, CTR; febrile seizures, FS; and septic meningitis, SM. Center lines denote medians, boxes denote 25–75% percentiles, and whiskers denote minimum and maximum values (white circles denote outliers). Parameters with statistically significant differences are noted with asterisks ($P < 0.05$) or double asterisks ($P < 0.01$).

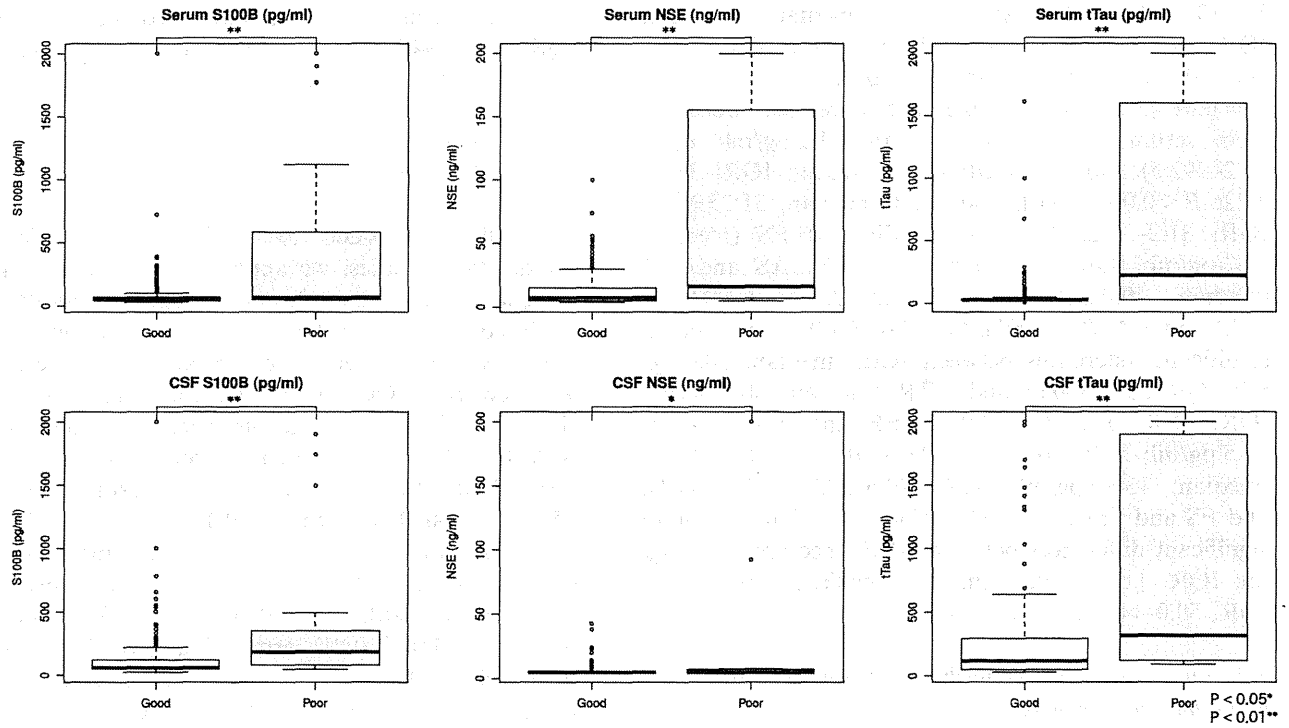

Fig. 2. Boxplot of serum (top) and CSF (bottom) levels of S100B (left), NSE (middle), and tTau (right) from patients with good prognosis (mRS 1–2) and poor prognosis (mRS 3–4). The centerlines denote medians, boxes denote 25–75% percentiles, and whiskers denote minimum and maximum values (white circles denote outliers). Parameters with statistically significant differences are noted with asterisks ($P < 0.05$) or double asterisks ($P < 0.01$).

Please cite this article in press as: Shiihara T et al. Serum and CSF biomarkers in acute pediatric neurological disorders. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.06.011

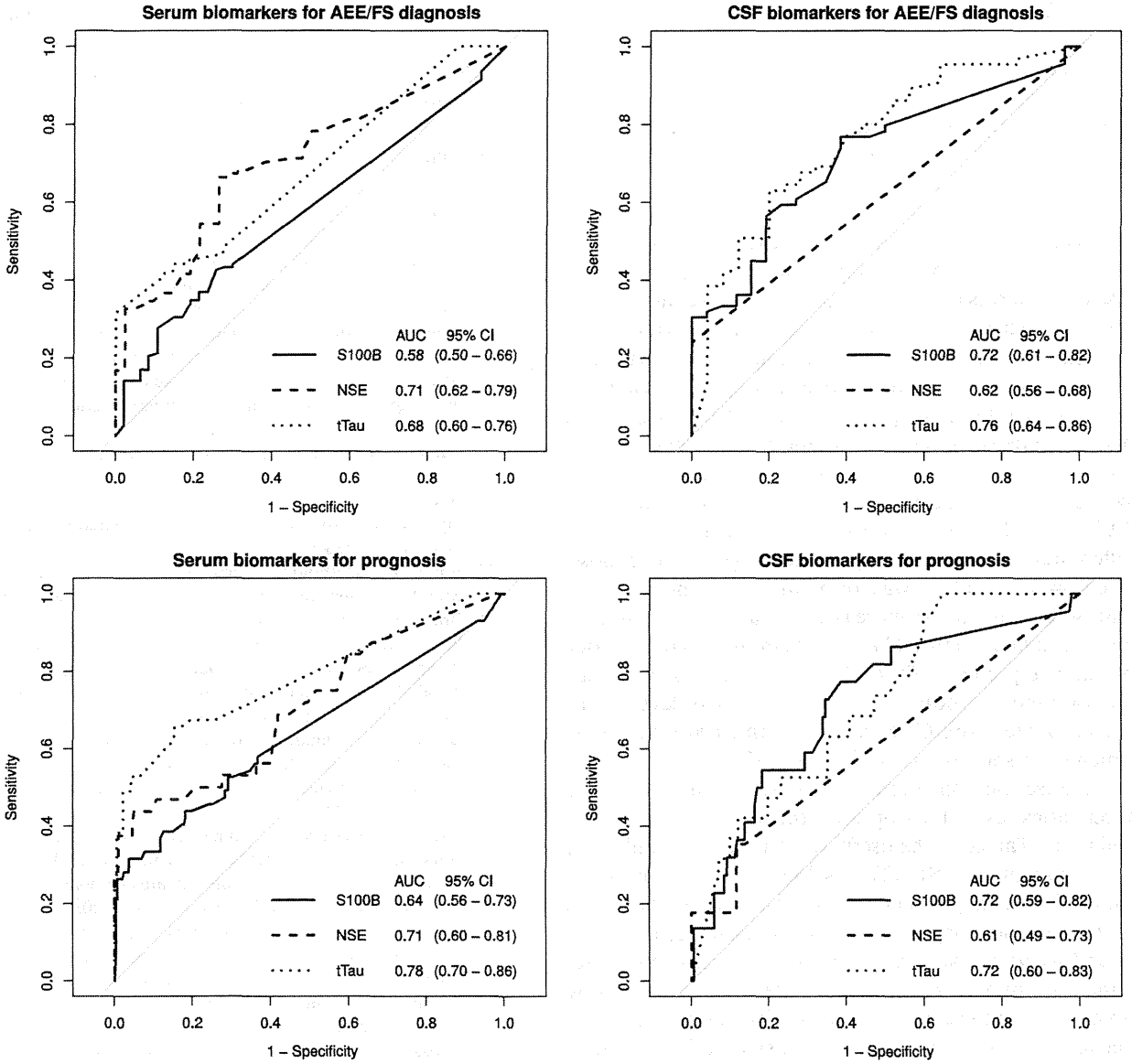

Fig. 3. ROC curves for diagnosis (top) and prognosis (bottom) with serum (left) and CSF (right) biomarkers (solid line, S100B; dashed line, NSE; and dotted line, tTau). AUCs are noted in the right lower corner with 95% CIs.

63.0% (95% CI 52.3–73.9%), and specificity 80.0% (95% CI 64.0–96.0%). The values for serum tTau (AUC 0.78) for distinguishing between poor and good prognoses were 68.8 pg/ml (Youden index 0.50), sensitivity 65.3% (95% CI 51.0–77.6%), and specificity 84.8% (95% CI 79.6–89.5%).

4. Discussion

There have been a many reports regarding the use of serum or CSF biomarkers to monitor various disorders, such as acute ischemic stroke, cerebral hemorrhage, traumatic brain injury, hypoxic ischemic encephalopathy, encephalitis, and meningitis [2–9]. Because different biomarkers were employed across studies and for different disorders, it was challenging to compare findings and establish firm conclusion; however, many biomarkers seemed to be increased in more severe disorders. Alterations in various biomarkers might reflect each central nervous system (CNS) cell damage rather than disease-specific changes. Here, we employed S100B, NSE, and tTau, as astrocytic, neuronal, and axonal damage markers in various acute pediatric neurological disorders to clarify their utility in making more precise diagnostic or prognostic predictions.

There were significant differences in serum NSE, serum tTau, CSF S100B, and CSF tTau (Fig. 1). As a whole, there tended to be the higher levels of the assessed biomarkers levels in AEE, which could reflect

Please cite this article in press as: Shiihara T et al. Serum and CSF biomarkers in acute pediatric neurological disorders. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.06.011

greater CNS damage than other more benign disorders. The level of serum NSE was higher in CTR than AS, however, their CSF NSE levels were not different. The patients in CTR were not healthy controls and NSE is also secreted outside CNS [3,16]. Thus the increased level of serum NSE in CTR must be reflected their extra CNS pathologies. As a prognostic evaluation, all biomarker levels were higher in patients with poor prognoses than in those with good prognoses (Fig. 2).

AEE can resemble FS, especially in an early stage of disease, in terms of fever, seizure, and consciousness disturbance. Therefore, we performed ROC curve analyses not only to differentiate between diagnoses of AEE and FS, but also to distinguish between poor and good prognoses. When AUC is higher than 0.75, the discriminative performance is thought to be good, and when AUC is higher than 0.90, it is thought to be excellent [11,12]. We found that CSF tTau was useful for discriminating AEE from FS (AUC = 0.76), and Serum tTau could differentiate between poor and good prognoses (AUC = 0.78). tTau was originally examined in CSF and was found to be increased in various neurological disorders; later, serum tTau was demonstrated as a good prognostic predictor [2–4,17–21]. Our results emphasize the usefulness of both CSF and serum tTau levels. tTau is considered more CNS specific than S100B and NSE, which corresponds to our findings [22].

Because they have relative low sensitivities and high specificities, as well as optimal threshold values, serum and CSF tTau could be useful for "ruling in" conditions, i.e., if serum or CSF tTau is higher than a threshold value, the patient is likely to have the target state, more severe disorder, or a more grim prognosis [23].

In this study, we did not employ a strict protocol for sampling timing or frequency. Therefore, it was not possible to do longitudinal analyses with serial specimens. Because CSF sampling is more invasive, serial CSF sampling is impractical. However, blood sampling is less invasive, so serial sampling for serum tTau examinations in various disorders could be a good strategy for further research. Finally, we would like to mention that an obstacle for clinical utilization of these biomarkers was that we used ELISA kits, which would not be suitable for a clinical setting, especially for emergencies in a patient-by-patient manner. Thus, a more convenient way for measuring potentially useful biomarkers is clearly needed.

Acknowledgments

This study was supported by the Medical Research Program of Gunma Prefectural Government, Japan. We declare that we have no conflict of interest. We are extremely grateful to all the patients, their parents, and the doctors who participated in this study. We are also grateful to members of the Facebook Organization of R Users for Medical Statistics in Japan (available at: https://www.facebook.com/groups/forums.japan/) for advising statistical analyses.

References

[1] Fenichel GM. Clinical pediatric neurology. 6th ed. Philadelphia: Saunders; 2009.
[2] Yuksel D, Yilmaz D, Uyar NY, Senbil N, Gurer Y, Anlar B. Tau proteins in the cerebrospinal fluid of patients with subacute sclerosing panencephalitis. Brain Dev 2010;32:467–71.
[3] Shiihara T, Miyake T, Izumi S, Watanabe M, Kamayachi K, Kodama K, et al. Serum and cerebrospinal fluid S100B, neuron-specific enolase, and total tau protein in acute encephalopathy with biphasic seizures and late reduced diffusion: a diagnostic validity. Pediatr Int 2012;54:52–5.
[4] Tanuma N, Miyata R, Kumada S, Kubota M, Takanashi J, Okumura A, et al. The axonal damage marker tau protein in the cerebrospinal fluid is increased in patients with acute encephalopathy with biphasic seizures and late reduced diffusion. Brain Dev 2010;32:435–9.
[5] Borusiak P, Herbold S. Serum neuron-specific enolase in children with febrile seizures: time profile and prognostic implications. Brain Dev 2003;25:272–4.
[6] Rodríguez-Núñez A, Cid E, Rodríguez-García J, Camiña F, Rodríguez-Segade S, Castro-Gago M. Cerebrospinal fluid purine metabolite and neuron-specific enolase concentrations after febrile seizures. Brain Dev 2000;22:427–31.
[7] Oyazato Y, Shiihara T, Kusunoki S, Adachi M, Ohnishi N, Taniguchi H, et al. A case of anti-GA1 antibody-positive Fisher syndrome with elevated tau protein in cerebrospinal fluid. Brain Dev 2012;34:329–32.
[8] Oka M, Hasegawa S, Matsushige T, Inoue H, Kajimoto M, Ishikawa N, et al. Tau protein concentrations in the cerebrospinal fluid of children with acute disseminated encephalomyelitis. Brain Dev 2013, in press. doi: 10.1016/j.braindev.2012.11.013.
[9] Wunderlich MT, Lins H, Skalej M, Wallesch CW, Goertler M. Neuron-specific enolase and tau protein as neurobiochemical markers of neuronal damage are related to early clinical course and long-term outcome in acute ischemic stroke. Clin Neurol Neurosurg 2006;108:558–63.
[10] Cnossen MH, Aarsen FK, Akker SLJ, Danen R, Appel IM, Steyerberg EW. Paediatric arterial ischaemic stroke: functional outcome and risk factors. Dev Med Child Neurol 2010;52:394–9.
[11] Robin X, Turck N, Hainard A, Tiberti N, Lisacek F, Sanchez JC, et al. PROC: an open-source package for R and S+ to analyze and compare ROC curves. BMC Bioinformatics 2011;12:77.
[12] Ray P, Le Manach Y, Riou B, Houle TT. Statistical evaluation of a biomarker. Anesthesiology 2010;112:1023–40.
[13] Hoshino A, Saitoh M, Oka A, Okumura A, Kubota M, Saito Y, et al. Epidemiology of acute encephalopathy in Japan, with emphasis on the association of viruses and syndromes. Brain Dev 2012;34:337–43.
[14] Mizuguchi M, Yamanouchi H, Ichiyama T, Shiomi M. Acute encephalopathy associated with influenza and other viral infections. Acta Neurol Scand Suppl 2007;186:45–56.
[15] Kawano G, Oshige K, Syutou S, Koteda Y, Yokoyama T, Kim BG, et al. Benign infantile convulsions associated with mild gastroenteritis: a retrospective study of 39 cases including virological tests and efficacy of anticonvulsants. Brain Dev 2007;29:617–22.

Please cite this article in press as: Shiihara T et al. Serum and CSF biomarkers in acute pediatric neurological disorders. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.06.011

[16] Kleine TO, Benes L, Zöfel P. Studies of the brain specificity of S100B and neuron-specific enolase (NSE) in blood serum of acute care patients. Brain Res Bull. 2003;61:265–79.
[17] Okumus N, Turkyilmaz C, Onal EE, Atalay Y, Serdaroglu A, Elbeg S, et al. Tau and S100B proteins as biochemical markers of bilirubin-induced neurotoxicity in term neonates. Pediatr Neurol 2008;39:245–52.
[18] Noguchi-Shinohara M, Hamaguchi T, Nozaki I, Sakai K, Yamada M. Serum tau protein as a marker for the diagnosis of Creutzfeldt–Jakob disease. J Neurol 2011;258:1464–8.
[19] Hu HT, Xiao F, Yan YQ, Wen SQ, Zhang L. The prognostic value of serum tau in patients with intracerebral hemorrhage. Clin Biochem 2012;45:1320–4.
[20] Protas PT, Muszynska-Roslan K, Holownia A, Grabowska A, Wielgat P, Krawczuk-Rybak M, et al. Negative correlation between cerebrospinal fluid tau protein and cognitive functioning in children with acute lymphoblastic leukemia. Pediatr Blood Cancer 2009;53:105–8.
[21] Liliang PC, Liang CL, Lu K, Wang KW, Weng HC, Hsieh CH, et al. Relationship between injury severity and serum tau protein levels in traumatic brain injured rats. Resuscitation 2010;81:1205–8.
[22] Cata JP, Abdelmalak B, Farag E. Neurological biomarkers in the perioperative period. Br J Anaesth 2011;107:844–58.
[23] Akobeng AK. Understanding diagnostic tests 1: sensitivity, specificity and predictive values. Acta Paediatr 2007;96:338–41.

Please cite this article in press as: Shiihara T et al. Serum and CSF biomarkers in acute pediatric neurological disorders. Brain Dev (2013), http://dx.doi.org/10.1016/j.braindev.2013.06.011

Epilepsia, **(*):1–7, 2013
doi: 10.1111/epi.12168

SPECIAL REPORT

SCN1A testing for epilepsy: Application in clinical practice

***Shinichi Hirose, †Ingrid E. Scheffer, ‡Carla Marini, §¶Peter De Jonghe, #Eva Andermann, **Alica M. Goldman, ††Marcelo Kauffman, ‡‡Nigel C. K. Tan, §§Daniel H. Lowenstein, ¶¶##Sanjay M. Sisodiya, ***Ruth Ottman, †††Samuel F. Berkovic, and for the Genetics Commission of the International League Against Epilepsy**

*Department of Pediatrics and Research Institute for the Molecular Pathomechanisms of Epilepsy, Fukuoka University, Fukuoka, Japan; †Florey Institute, Departments of Medicine and Paediatrics, Austin Health and Royal Children's Hospital, University of Melbourne, Melbourne, Victoria, Australia; ‡Epilepsy, Neurophysiology and Neurogenetics Unit, Division of Child Neurology and Psychiatry, University of Pisa and Research Institute Stella Maris Foundation, Pisa, Italy; §Neurogenetics Group, VIB-Department of Molecular Genetics and Laboratory of Neurogenetics, Institute Born-Bunge, University of Antwerp, Antwerpen, Belgium; ¶Department of Neurology, Antwerp University Hospital, Antwerpen, Belgium; #Neurogenetics Unit, Montreal Neurological Hospital & Institute, Montreal, Quebec, Canada; **Department of Neurology, Baylor College of Medicine, Houston, Texas, U.S.A.; ††Neurogenetics Clinic Hospital JM Ramos Mejia, University of Buenos Aires-CONICET, Buenos Aires, Argentina; ‡‡Department of Neurology, National Neuroscience Institute, Singapore, Singapore; §§Department of Neurology, University of California, San Francisco, California, U.S.A.; ¶¶UCL Institute of Neurology, London, United Kingdom; ##Epilepsy Society, Buckinghamshire, United Kingdom; ***Sergievsky Center and Departments of Epidemiology and Neurology, Columbia University, New York, New York, U.S.A.; and †††Epilepsy Research Centre, University of Melbourne, Austin Health, Melbourne, Victoria, Australia

Summary

This report is a practical reference guide for genetic testing of *SCN1A*, the gene encoding the α1 subunit of neuronal voltage-gated sodium channels (protein name: $Na_v1.1$). Mutations in this gene are frequently found in Dravet syndrome (DS), and are sometimes found in genetic epilepsy with febrile seizures plus (GEFS+), migrating partial seizures of infancy (MPSI), other infantile epileptic encephalopathies, and rarely in infantile spasms. *Recommendations for testing:* (1) Testing is particularly useful for people with suspected DS and sometimes in other early onset infantile epileptic encephalopathies such as MPSI because genetic confirmation of the clinical diagnosis may allow optimization of antiepileptic therapy with the potential to improve seizure control and developmental outcome. In addition, a molecular diagnosis may prevent the need for unnecessary investigations, as well as inform genetic counseling. (2) *SCN1A* testing should be considered in people with possible DS where the typical initial presentation is of a developmentally normal infant presenting with recurrent, febrile or afebrile prolonged, hemiclonic seizures or generalized status epilepticus. After age 2, the clinical diagnosis of DS becomes more obvious, with the classical evolution of other seizure types and developmental slowing. (3) In contrast to DS, the clinical utility of *SCN1A* testing for GEFS+ remains questionable. (4) The test is not recommended for children with phenotypes that are not clearly associated with *SCN1A* mutations such as those characterized by abnormal development or neurologic deficits apparent at birth or structural abnormalities of the brain. *Interpreting test results:* (1) Mutational testing of *SCN1A* involves both conventional DNA sequencing of the coding regions and analyses to detect genomic rearrangements within the relevant chromosomal region: 2q24. Interpretation of the test results must always be done in the context of the electroclinical syndrome and often requires the assistance of a medical geneticist, since many genomic variations are possible and it is essential to differentiate benign polymorphisms from pathogenic mutations. (2) Missense variants may have no apparent effect on the phenotype (benign polymorphisms) or may represent mutations underlying DS, MPSI, GEFS+, and related syndromes and can provide a challenge in interpretation. (3) Conventional methods do not detect variations in introns or promoter or regulatory regions; therefore, a negative test does not exclude a pathogenic role of *SCN1A* in a specific phenotype. (4) It is important to note that a negative test does not rule out the clinical diagnosis of DS or other conditions because genes other than *SCN1A* may be involved. Obtaining written informed consent and genetic counseling should be considered prior to molecular testing, depending on the clinical situation and local regulations.

KEY WORDS: Diagnosis, Epileptic encephalopathy, Guideline, Seizures, severe myoclonic epilepsy in infancy (SMEI), Dravet syndrome, Sodium channel.

Genetic testing has become a powerful tool in clinical epilepsy practice in certain situations. In particular, analyses targeting the gene encoding the α1 subunit of the neuronal

Accepted March 4, 2013.

Address correspondence to Shinichi Hirose, Department of Pediatrics, Fukuoka University, 45-1 7-chome Nanakuma, Jonan-ku Fukuoka, 814-0180 Japan. E-mail: hirose@fukuoka-u.ac.jp

voltage-gated sodium channel *SCN1A* (protein name: $Na_v1.1$), are clinically valuable in confirming the clinical diagnosis of Dravet syndrome (DS). Mutations of *SCN1A* may also be found as a cause of genetic epilepsy with febrile seizures plus (GEFS+), migrating partial seizures of infancy (MPSI), and rarely in other syndromes. Mutations of *SCN1A* are found in 70–80% of patients with DS and in up to 10% of families with GEFS+ (Scheffer & Berkovic, 2000). The purpose of this report is to provide clinicians with practical guidance for *SCN1A* gene testing. We discuss the Who, Why, What, Where, and How of testing.

Who

Who may have a positive *SCN1A* result?

Suspected Dravet syndrome (DS) is the principal indication for *SCN1A* testing, and 70–80% of cases have a demonstrable mutation. DS typically presents between 4 and 8 months of age (range: up to 15 months) with recurrent prolonged convulsive seizures that may be lateralized (hemiclonic) or generalized (Dravet, 1978; Dravet et al., 1982, 2002). Seizures are often associated with fever or occur shortly after vaccination, which has led to the misdiagnosis of "vaccine encephalopathy" (Berkovic et al., 2006; McIntosh et al., 2010). Myoclonic, focal, and atypical absence seizures may begin between 1 and 4 years. Infants with DS usually develop normally in the first year. Developmental stagnation or regression becomes evident in the second year of life and cognitive outcome is usually poor. It is important to note that the syndromic picture takes time to evolve and early recognition may be challenging (Dravet, 1978; Dravet et al., 1982, 2002).

DS should be considered in infants with febrile seizures (FS) presenting around 6 months of age, especially those with prolonged and recurrent FS (Hattori et al., 2008; Millichap et al., 2009; Fountain-Capal et al., 2011), hemiclonic seizures, and seizures induced by bathing (Oguni et al., 2001; Hattori et al., 2008).

Genetic testing of older patients with an early history consistent with DS helps to confirm the diagnosis of DS, which may have been missed due to the relatively recent recognition of the syndrome or because of difficulties in obtaining a clear early history. Providing a molecular diagnosis is often extremely helpful to the family because it gives them an understanding of the etiology of their relative's epilepsy and intellectual disability. It also informs the prognosis and is key for genetic counseling for family members. Furthermore, recent evidence suggests that optimization of treatment even in later adult life may improve cognition (Catarino et al., 2011).

Individuals with MPSI

Infants with MPSI present with multiple types of focal seizures that begin in early infancy and increase in frequency and prove highly refractory to antiepileptic therapy. The key electroclinical feature is interhemispheric migration during a seizure (Coppola et al., 1995). Electroencephalography (EEG) shows frequent multifocal epileptiform abnormalities. MPSI is more severe than DS, with profound developmental impairment, and is considered one of the most severe forms of early infantile epileptic encephalopathy. The syndrome is much rarer than DS and easily distinguished from DS by its clinical presentation. The main cause appears to be mutations in *KCNT1* (Barcia et al., 2012), but *SCN1A* mutations have been found in a few cases (Carranza Rojo et al., 2011; Freilich et al., 2011). The strategy for genetic testing here is yet to be firmly established, but mutations in *KCNT1* should currently be considered first, and then *SCN1A* may be examined.

Individuals with other severe infantile epilepsies

SCN1A mutations may also be found in approximately 50% of children with disorders that bear some resemblance to DS. These include severe infantile multifocal epilepsy (SIMFE) (Harkin et al., 2007) and intractable childhood epilepsies with frequent generalized tonic–clonic seizures (Fujiwara et al., 2003).

SCN1A mutations are found in a small minority of children (5% or less) with other specific syndromic forms of infantile epilepsy including West syndrome (Wallace et al., 2003), generalized or focal epilepsies of unknown cause (Harkin et al., 2007), epilepsy with myoclonic-atonic seizures (previously called myoclonic-astatic epilepsy) (Wallace et al., 2001), and hemiconvulsion-hemiplegia syndrome (Sakakibara et al., 2009). Routine testing in patients with these syndromes is not currently recommended.

Individuals with epilepsy in families with GEFS+

GEFS+ is a familial syndrome usually with complex inheritance, but sometimes showing autosomal dominant transmission. Family members have extremely variable phenotypes, including typical febrile seizures, febrile seizures plus (FS+), and generalized and focal epilepsies (Scheffer & Berkovic, 1997; Wallace et al., 1998; Scheffer et al., 2009).

The clinical utility of *SCN1A* genetic testing for GEFS+ is limited because few families (approximately 10%) have been found to have mutations, and the identification of a mutation does not predict the phenotype that will develop in an individual (Ottman et al., 2010). At present, therefore, infants and children in families with GEFS+ should not be advised to undergo *SCN1A* testing as it will neither influence management nor provide information regarding the patient's prognosis.

Who is unlikely to harbor an *SCN1A* mutation?

SCN1A mutations are not found in children with confirmed metabolic disorders, genetic syndromes, or those with structural abnormalities of the brain. For a child with

an epileptic encephalopathy with features not found in DS, such as neonatal onset, or developmental delay prior to seizure onset, *SCN1A* testing is unlikely to be helpful. A variety of other genes have been associated with early onset epileptic encephalopathies, including *ARX, STXBP1, and CDKL5* where developmental delay and interictal epileptiform abnormalities in early infancy are usual.

Another differential diagnosis to be considered is epilepsy and mental retardation limited to females (EFMR), perhaps better denoted *PCDH19*-female-limited epilepsies (as not all affected females have mental retardation (Dibbens et al., 2008; Scheffer et al., 2008), which share phenotypic features with DS (Depienne et al., 2009). However, status epilepticus induced by fever is a rare clinical presentation in this syndrome, which presents with clusters of brief febrile seizures continuing over several days (Marini et al., 2010; Higurashi et al., 2011). *PCDH19*-female-limited epilepsies are less likely to be associated with myoclonic and absence seizures, more likely to be associated with autistic features, and carry a better intellectual prognosis than does DS (Dibbens et al., 2008; Scheffer et al., 2008). *PCDH19*-female-limited epilepsies have a distinctive inheritance pattern as only females are affected, so recognition of this pattern in a family suggests that *PCDH19* may be more appropriate for initial testing than *SCN1A*.

Why

Why is *SCN1A* testing useful in DS?

Molecular confirmation of *SCN1A* defects supports the clinical diagnosis and is of considerable importance for genetic counseling. Knowledge of the gene involved will guide the selection of antiepileptic treatment. Some antiepileptic drugs such as specific sodium channel blocking agents, for example, carbamazepine and lamotrigine, are contraindicated as they may aggravate symptoms (Guerrini et al., 1998), whereas stiripentol has been shown to be effective in children (Chiron et al., 2000). Anecdotal evidence suggests that the early use of appropriate drugs, and avoidance of medications that may worsen DS, may lead to an improved long-term outcome, but this needs more rigorous assessment.

When an *SCN1A* mutation is identified, the parents must be investigated for this particular mutation to establish if it has arisen de novo. Among *SCN1A* mutations identified in patients with DS, 90% are de novo (Depienne et al., 2009). The remaining 10% of identified mutations are inherited and family members often have milder GEFS+ phenotypes. It is notable that there are now many well-documented instances of mosaicism (i.e., a mixture of mutation-carrying and noncarrying cells) in the parents of DS patients, either in the germ cells (parental germ line mosaicism) or somatic cells (parental somatic mosaicism). Both forms of parental mosaicism markedly increase the risk of parents having a second child with DS (Depienne et al., 2006). Also notable is that the percentage of abnormal cells in the mosaic parent correlates with whether the parent is affected and the severity of the parent's epilepsy (Depienne et al., 2010). These observations highlight the importance of genetic counseling for families with DS or another *SCN1A*-related epileptic encephalopathy.

What

What do the tests mean?

Comprehensive evaluation for an *SCN1A* mutation requires that two different testing methods be performed (Table 1). Conventional DNA sequencing has the highest yield and should be carried out first to screen the gene's coding regions and associated splice junctions. The approach is based on classic PCR (polymerase chain reaction) methodology followed by Sanger sequencing. Because directed sequencing is limited in coverage and commonly restricted to gene regions likely to affect the encoded protein and previously identified regulatory regions (the coding sequence, all splice junctions, and—in rarer cases—the 5′ and 3′ untranslated regions as well as the proximal and distal promoter, mutations in areas of apparently lesser relevance can be overlooked (Nakayama et al., 2010). Second, multiplex ligation-dependent probe amplification (MLPA) or comparative genomic hybridization (CGH) (Mulley et al., 2006; Suls et al., 2006; Wang et al., 2008; Marini et al., 2009) should be carried out to detect genomic rearrangements, such as microdeletions or microduplications within *SCN1A* and also in the *SCN1A* gene neighborhood of chromosomal region 2q24.

Table 2 explains mutation nomenclature. Truncations of the α1 subunit of $Na_v1.1$, which often result from nonsense mutations, splice site mutations, and small insertions and deletions (indels), are likely to have considerable impact on the function of $Na_v1.1$ and hence to cause severe phenotypes such as DS or MPSI. A missense mutation is a single nucleotide mutation that alters only one amino acid. Compared with truncation mutations, missense mutations generally have less impact on the $Na_v1.1$ function but nevertheless are responsible for about half the cases of DS, so the nature of the mutation does not allow the clinician to confidently predict the phenotype (Zuberi et al., 2011). The *SCN1A* mutations identified in GEFS+ have been largely missense mutations. Missense mutations altering the polarity of the amino acids in the pore-forming and voltage-sensor regions may be more likely to have a severe phenotype (Zuberi et al., 2011).

Genomic rearrangements, typically chromosomal microdeletions and microduplications, may involve several genes contiguous to *SCN1A* and hence result in severe forms of DS and other epilepsies, but sometimes with more extensive features involving other systems.

The *SCN1A* mutations identified are heterozygous, affecting one allele (either the maternal or the paternal gene

Table 1. *SCN1A* mutations and interpretations

Type of variations	Common phenotypes	Uncommon/rare phenotypes	Explanatory examples for the description (Lossin, 2009)
Small scale variations			
Missense mutation	DS, GEFS+	MPSI, SIMFE, ICEGTC	c.3820T>A: p.Y1274N c.5075T>C: p.F1692S
Nonsense mutation	DS	SIMFE	c. 1834 C>T: p.R612X c. 3858 G>A: p.W1286X
Splice-site mutation	DS		c.265-1G>A c.1662+1G>T
Deletion mutation	DS	MPSI	c.429_430delGT: p.V143VfsX149 c.5296_5298delTTT: p.F1766del
Insertion mutation	DS		c.992_993insT: p.L331fsX340 c.1640_1641insA: p.K547fsX549
Large-scale variations			
Microdeletion	DS		
Microduplication	DS	SGE	
cf) Nonpathogenic small-scale variations			
Polymorphism	Nonpathogenic coding variant found in general population	c.1662G>A: p.Q554Q c.5771G>A: p.R1924H	

DS, Dravet syndrome; MPSI, migrating partial seizures of infancy; GEFS+, genetic epilepsy with febrile seizures plus; SIMFE, severe infantile multifocal epilepsy; ICEGTC, intractable childhood epilepsy with generalized tonic–clonic seizures, SGE, symptomatic generalized epilepsy.

(1) Small-scale *SCN1A* variations

Missense mutations are due to single nucleotide exchange resulting in a substitution of a single amino acid. This exchange suggests protein dysfunction using evidence drawn from in vitro or in vivo testing or in silico analysis.

Nonsense mutations generate premature stop codons truncating the α1 subunit molecule, thus named also "truncation mutation." The truncation mutations generally result in DS or related epileptic encephalopathies. They are designated by adding an "X" at the end of their descriptions, for example, c.C1834T: p.R612X, c.429-430delGT: p.V143VfsX149.

Splice-site mutations are located in the vicinity of a splice junction at the intron/exon boundaries. They affect the messenger RNA (mRNA) and lead to considerable changes in the α1 subunit amino acid content (e.g., exon skipping, nonsensical translation of introns) that commonly result in premature truncation due to stochastic occurrence of a stop codon. Determining the effect of splicing abnormalities is challenging and usually exceeds the scope of standard genetic analyses. As such, typical genetic testing will comment only on those splice-site mutations that have been previously extensively studied in a laboratory and have proven functional impact.

Deletions of base pairs have one of two consequences: (1) an in-frame deletion eliminates one or more amino acids from the α1 subunit molecule; (2) a frame-shift mutation recodes all residues downstream of the variation site and frequently results in a premature stop codon. As such they severely impair protein function. Several meta-analyses attempting to identify *SCN1A* genotype–phenotype correlations have found evidence for higher seizure severity with structurally/functionally more significant changes in the $Na_v1.1$ protein.

Insertions are analogous to deletions. They add contiguous nucleotides in the *SCN1A* gene. The consequences are also similar to that of deletion mutations, commonly resulting in DS.

(2) Large-scale *SCN1A* variations

Variations of this kind commonly affect the copy number of the *SCN1A* gene as they involve chromosomal rearrangements that may delete or duplicate entire gene regions.

Microdeletions: Approximately 10% of all individuals for whom conventional sequence analysis of *SCN1A* does not reveal any abnormalities harbor microdeletions that may affect not only *SCN1A* but also adjacent genes (Wang et al., 2008; Marini et al., 2009). Microdeletions typically cause DS, even if adjacent genes (e.g., *SCN2A*, *SCN7A*, etc.) are involved (Wang et al., 2008).

Microduplications: Chromosomal duplications ranging from 1 kb to several Mbp are a rare cause of DS (Marini et al., 2009; Heron et al., 2010; Raymond et al., 2011).

Table 2. Checklist for *SCN1A* genetic test

Explain the potential benefit and harms of the test
Disclose limitations of the test
Provide genetic counseling
Obtain written informed consent depending on local regulations

copy). As noted above, in DS most *SCN1A* mutations are de novo, occurring for the first time as the result of a genetic event either in the germ cell line of one of the parents or during early embryogenesis, since neither parent has the mutation. The possibility of germ line or somatic mosaicism needs to be considered (see above). Detection of *SCN1A* mosaicism is often difficult, especially when it affects the germ line, which complicates genetic counseling for DS (Depienne et al., 2006; Gennaro et al., 2006; Marini et al., 2006). In contrast, cases of GEFS+ with *SCN1A* mutations are usually familial, although de novo cases are known. Occasionally, a patient with DS may be observed in a family where other members have GEFS+ with mild seizure disorders. All the affected family members have the familial *SCN1A* mutation and it is presumed that the more severely affected family member with DS has additional genetic variants that contribute to their severe phenotype.

Databases listing all currently reported variations are available: *The SCN1A Infobase* (http://www.scn1a.info/)