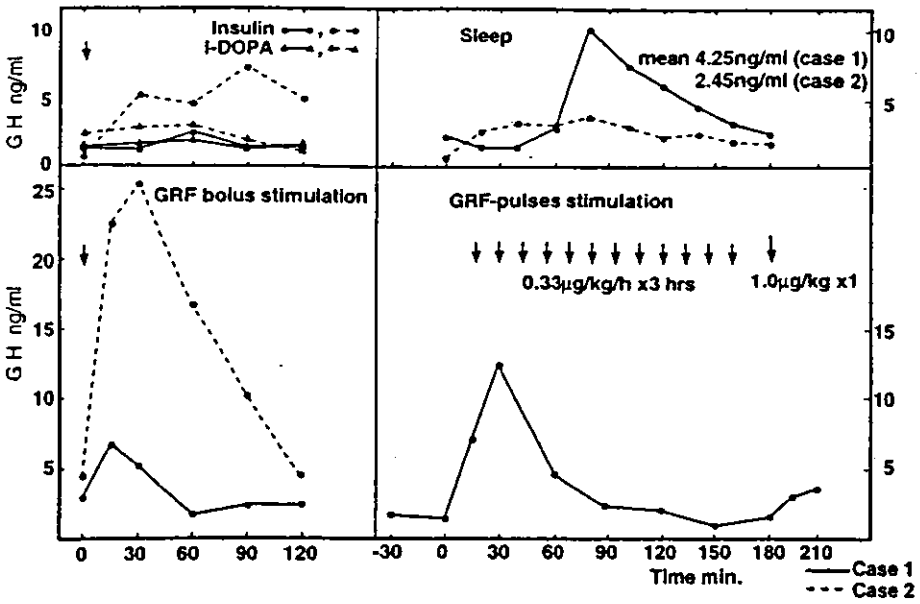

Fig. 1 GH secretion tests. GH secretion in the presence of insulin, I-DOPA and sleep showed a notably deficient pattern. GH secretion was normal in case 2 with a single dose of GRF, and was normal in case 1 with stronger pulsating GRF stimulation.

tial seizures and mild psychomotor retardation, with elevated serum levels of lactate and pyruvate confirmed under the exercise-loading test. Ragged red fibers were not apparent in a muscle biopsy performed at 3 years of age. Her height was 116.2 cm (– 0.8 SD) at the age of 7 years 11 months, and was stunted at 126.4 cm at 10 years 3 months of age (– 1.8 SD) with a bone age of 10 years.

Case 2

This boy was the only child of non-consanguineous healthy Japanese parents. He was born at 32 weeks gestation after an uncomplicated pregnancy and birth, weighing 2225 g, an appropriate weight for the term of the pregnancy. He suffered from respiratory distress in the neonatal period, and was assisted with mechanical ventilation. Psychomotor development and physical growth were normal. At 3 years 5 months of age, he presented with fatigability and generalized muscle weakness. He developed frequent episodes of headaches, vomiting, complex partial seizures and transient visual impairment from the age of 4 years 6 months. His height was 88.2 cm (– 1.4 SD) at 3 years of age, and was 92.1 cm (– 2.0 SD) at 4 years of age with a bone age of 3 years 6 months.

Results

Serum levels of lactate and pyruvate were elevated in both cases. Lactate levels were 28.7 mg/dl and 51.1 mg/dl for cases 1 and 2, and pyruvate levels were 0.98 mg/dl and 1.55 mg/dl for case 1 and case 2, respectively. Lactic acidosis was confirmed in both cases. In case 2 the serum levels of CK and LD were elevated to 484 IU/l and 1028 IU/l, respectively. Case 2 was diagnosed with mild dilated cardiomyopathy at 6 years 4 months of age. Muscle biopsies were performed with the informed and written consents of parents and demonstrated the presence of abundant ragged red fibers in case 1 at 7 years 11 months and in case 2 at 3 years 6 months. The activities of respiratory chain enzymes of complex I – IV in muscle were all within the normal range in both cases. The DNA sample was prepared from the muscle biopsy taken from case 2 only. A point mutation A3243 G was identified. Lactic acidosis was diagnosed in the mother of case 2, and a muscle biopsy identified ragged red fibers. In case 1 at 7 years 9 months of age a brain CT revealed a low-density area in the right temporal cortex, the left temporal to posterior temporal cortex and in the bilateral globus pallidus. Diffuse brain atrophy then progressed rapidly. Case 2 showed low-density areas in the left occipital lobe. Frontal dominant diffuse progressive brain atrophy and high-density areas in the bilateral globus pallidus were revealed at 6 years 1 month of age. In both cases CT did not detect the localized hypothalamic lesions.

Endocrinological functions were evaluated at 10 years 3 months for case 1, and at 4 years 0 month for case 2. Serum IGF-I levels were 1.3 U/ml for case 1 and 0.25 U/ml for case 2. The peak levels of GH with insulin loading were 2.4 ng/ml for case 1 and 7.3 ng/ml for case 2, and with I-DOPA were 1.9 ng/ml for case 1 and 3.0 ng/ml for case 2. The mean GH values during sleep were 4.25 ng/ml for case 1 and 2.45 ng/ml for case 2. On administration of a single loading of GRF 1 μg/kg, the peak level of GH was 6.7 ng/ml for case 1 and 25.4 ng/ml for case 2. In case 1, a normal GH response of 12.4 ng/ml was observed with pulsating GRF stimulation. This entailed intravenous administration of a low dose of GRF at 0.33 μg/kg/hr using pulsed injections every 15 min for 3 hours followed by a final bolus injection of GRF at 1.0 μg/kg [6] (Fig. 1). The peak levels of TSH with TRH loading were 17.8 μU/ml for case 1 and 10.0 μU/ml for case 2. The peak levels of cortisol with insulin loading were 24.5 μU/dl for case 1 and 21.1 μU/dl for case 2.

Recombinant hGH was administered at 0.17 mg/kg/week in case 1 for 2 years and 3 months from the age of 10 years 6 months, and in case 2 for 5 months from the age of 4 years 3 months, with the informed consent of parents. GH supplementation generated positive growth responses in both cases. In case 1 physical growth accelerated from 4.7 to 8.8 cm/year, and in case 2 from 5.5 to 11.1 cm/year, without any change in serum levels of CK and LD during the period of GH supplementation.

Discussion

The variety of clinical manifestation of mitochondrial cytopathy may depend on the heteroplasmy of mutant mitochondrial DNA and its tissue-specific distribution, in addition to the dependence of individual tissues on mitochondrial energy production [11]. Despite short stature being a common symptom of mitochondrial cytopathy, investigations into GH deficiency, the underlying primary lesion for short stature, and the clinical effectiveness of GH supplementation are limited [1,5]. We confirmed GH deficiency in the patients using several GH secretion stimulation tests and also confirmed that the GRF producing cells of the hypothalamus might be the site of the primary lesion, not the GH producing cells in the anterior pituitary lobe. Case 1 showed the normal GH response only after repetitive GRF stimulation, which might be related to the prolonged absence of GRF stimulation. As the other anterior pituitary hormones yielded normal secretion patterns, it is possible that the GRF producing cells in the arcuate and ventromedial nuclei [12] are more vulnerable to lactic acidosis than the cells producing GH and other hormones in the anterior and posterior lobes of the pituitary, or the endocrine cells of the hypothalamus. Tissue vulnerability may be related to the tissue specific distribution of mtDNA. The difference in GRF response in these two cases might be due to the age of the patient and duration of metabolic acidosis.

Ohama reported that vascular lesions in the smooth muscle and endothelial cells of cerebral vessels originated primarily as a result of mitochondrial dysfunction and were referred to as "mitochondrial angiopathy". Abnormal mitochondria and a marked reduction in GH secretory granules appear in the lobe cells of the anterior pituitary [8], and accumulation of mitochondria is most prominent in the walls of pial arterioles and small arteries [9]. These arteries might function to autoregulate cerebral blood flow. Mitochondrial microangiopathy in the hypothalamus may suggest the correspondence to our endocrinological study.

Adverse effects and exacerbation of epileptic seizures, stroke-like episodes, metabolic acidosis and elevated serum levels of CK and LD during GH supplementary therapy were not observed in these two cases. Egger et al [5] described the effectiveness of GH supplementation but did not note any adverse effects. Patients with long-term GH deficiency have an increased risk of mortality due to cardiovascular disease [2]. For this reason determining the optimal dose for GH supplementation is important. Colao et al [3] reported that supplementation with GH at 10–20 µg/kg in young adult patients with GH deficiency improved cardiac vascular risk. This is consistent with the dose used for the cases described in this report. The long-term effects of GH supplementation for patients with MELAS are unknown. Critical evaluation of long-term GH therapy may elucidate the adverse effects and efficacy of such treatment.

References

[1] Burns EC, Preece MA, Dameron N, Tanner JM. Growth hormone deficiency in mitochondrial cytopathy. Acta Paediatr Scand 1982; 71: 693–697

[2] Carroll PV, Christ ER and the members of Growth Hormone Research Scientific Committee. Growth hormone deficiency in adulthood and the effects of growth hormone replacement: a review. J Clin Endocrinol Metab 1998; 83: 382–395

[3] Colao A, Di Somma C, Cuocolo A, Spinelli L, Tedesco N, Pivonello R et al. Improved cardiovascular risk factors and cardiac performance after 12 months of growth hormone (GH) replacement in young adult patients with GH deficiency. J Clin Endocrinol Metab 2001; 86: 1874–1881

[4] DiMauro S, Bonilla E, Zviani M, Nakagawa M, DeVivo DC. Mitochondrial myopathies. Ann Neurol 1985; 17: 521–538

[5] Egger J, Lake BD, Wilson J. Mitochondrial cytopathy. A multisystem disorder with ragged red fibres on muscle biopsy. Arch Dis Child 1981; 56: 741–752

[6] Kopelman PG, Noonan K. Growth hormone response to low dose intravenous injections of growth hormone releasing factor in obese and normal weight women. Clin Endocrinol 1986; 24: 157–164

[7] Mosewich RK, Donat JR, DiMauro S, Ciafaloni E, Shanske S, Erasmus M et al. The syndrome of mitochondrial encephalomyopathy, lactic acidosis, and stroke-like episodes presenting without stroke. Arch Neurol 1993; 50: 275–278

[8] Ohama E. Neuropathology in MELAS. Jpn J Pediatr Med (in Jpn) 1991; 23: 1084–1089

[9] Ohama E, Ohara S, Ikuta F, Tanaka K, Nishizawa M, Miyatake T. Mitochondrial angiopathy in cerebral blood vessels of mitochondrial encephalopathy. Acta Neuropathol 1987; 74: 226–233

[10] Pavlakis SG, Phillips PC, DiMauro S, DeDivo DC, Rowland LP. Mitochondrial myopathy, encephalopathy, lactic acidosis and stroke-like episodes: a distinctive clinical syndrome. Ann Neurol 1984; 16: 481–488

[11] Reardon W, Ross RJM, Sweeney MG, Luxon LM, Pembrey ME, Harding AE et al. Diabetes mellitus associated with a pathogenic point mutation in mitochondrial DNA. Lancet 1992; 340: 1376–1379

[12] Reichlin S. Neuroendocrinology. In: Wilson JD, Foster DW, Kronenberg HM, Larsen PR (Eds). Williams Textbook of Endocrinology. 9th ed. Philadelphia: Saunders, 1998: 165–248

[13] Simpoulus AP, Delea CS, Bartler FC. Neurodegenerative disorders and hyperaldosteronism. J Pediatr 1971; 79: 633–641

[14] Toppett M, Tolerman-Toppett N, Szliwowski HB, Bainsel M, Coers C. Oculocraniosomatic neuromuscular disease with hypoparathyroidism. Am J Dis Child 1977; 131: 437–441

X. てんかん症候群　その他の重要な病態

てんかん性陰性ミオクローヌスを伴う非けいれん性てんかん重積状態*

大塚頌子[1]

1. 概　念

1983年，大田原ら[1]，および著者ら[2]が'小児期における非けいれん性てんかん重積状態の特異型'(peculiar type of nonconvulsive status epilepticus in childhood)として報告したものに基づき，後に大田原が'てんかん性陰性ミオクローヌスを伴う非けいれん性てんかん重積状態'(nonconvulsive status epilepticus with epileptic negative myoclonus: NSENM)と名付けた[3]．徐波睡眠時に持続性棘徐波を示すてんかん(epilepsy with continuous spike-waves during slow wave sleep: ECSWS[4])に近縁の状態で，脳波上睡眠時に広汎性棘徐波が持続的に出現するが，臨床的には短い脱力のエピソード(atonic episodes)の群発と一過性の一側性運動障害を特徴とする．atonic episodesにつき，発表当時は脳波上の棘徐波と一対一の関係が明確でないと考え，狭義のてんかん発作ではないと記載したが，後に詳しい脳波・筋電図同時記録の分析により，これはてんかん性陰性ミオクローヌス(ENM)[5,6]であると結論するに至った(図1)．

2. 分　類

ILAEのてんかんおよびてんかん症候群分類[7]では，ECSWSは焦点性か全般性か決定できないてんかんおよびてんかん症候群に分類されている．NSENMはECSWSに近縁の病型であるが，発作はENMを含め，基本的に部分発作と考えられる．脳波では焦点性発射が多く，徐波睡眠時の広汎性棘徐波の持続的出現(CSWS)も焦点性発射の二次性全般化と見なされるため，局在関連性てんかんの特殊な状態と考えられる．

3. 病　因

基礎疾患を認めない症例が多いが，多小脳回，特にmultilobar polymicrogyriaを基盤に発症する症例がある．

4. 病　態

a. 臨床像

1) てんかん発作

てんかん発作の初発年齢は幼児期から学童期である．発作型は部分運動発作と二次性全般化発作が多く，主に睡眠時に認められる．それと同時期，多くは数カ月から数年を経て，4-7歳ごろ，特異な脱力のエピソード，すなわちENMが頻発するNSENMの状態になる．ENM自体は意識障害を伴わないが，NSENMの時期に非定型欠神を合併する症例もある．

2) てんかん性陰性ミオクローヌス

通常頻発し，四肢，頸部，上半身に多く認められ，腕の脱力や頭部前屈，坐位での上半身の前傾という形で出現する．特に，腕を前方に伸展した状態では，腕が何度も下垂するのがわかる．下肢に認められるときには，立位や歩行時

Key words：てんかん性陰性ミオクローヌスを伴う非けいれん性てんかん重積状態，てんかん性陰性ミオクローヌス，非けいれん性てんかん重積状態，徐波睡眠時に持続性棘徐波を示すてんかん，atypical benign partial epilepsy of childhood　*Nonconvulsive status epilepticus with epileptic negative myoclonus(NSENM)
[1]Yoko OHTSUKA: 岡山大学大学院医歯学総合研究科発達神経病態学　Department of Child Neurology, Okayama University Graduate School of Medicine and Dentistry

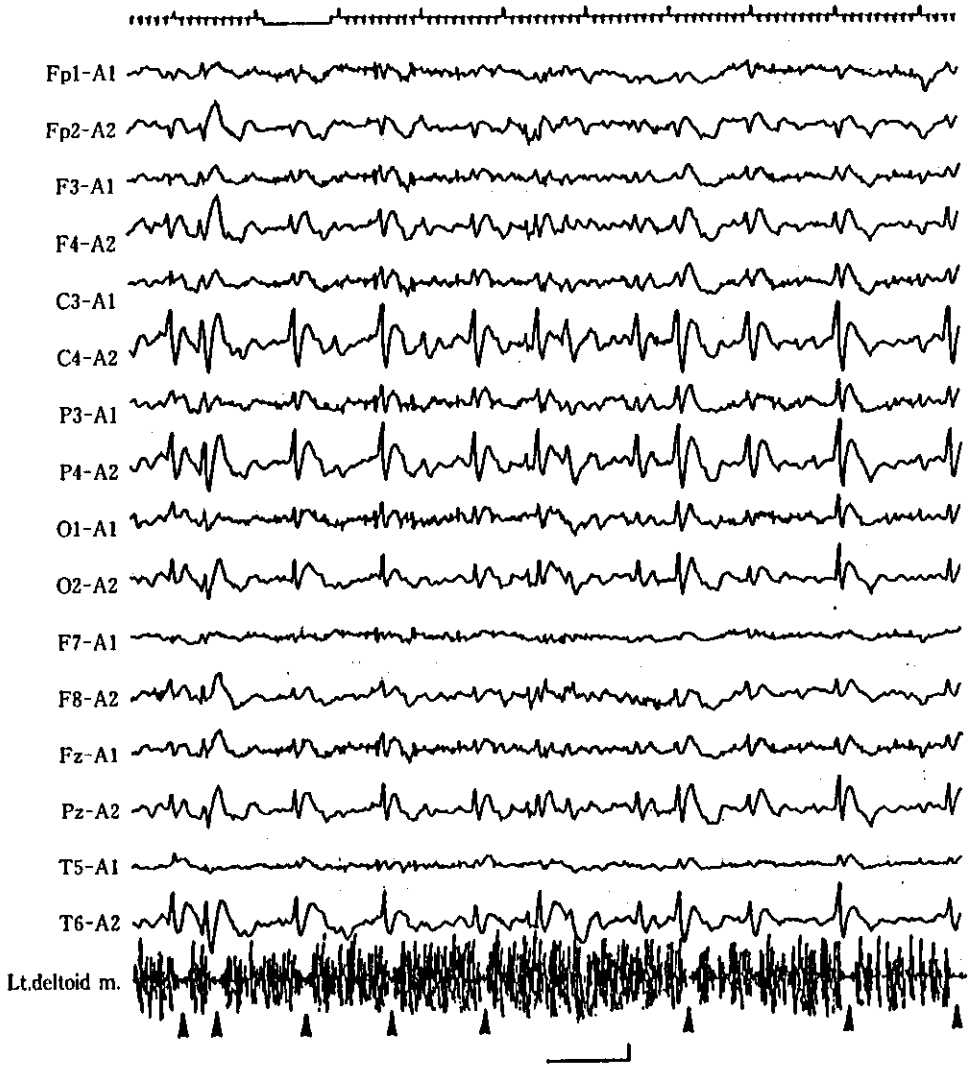

図1　NSENMで認められたてんかん性陰性ミオクローヌス

両上肢を前方に挙上させた状態で記録すると，左上肢が繰り返し下垂するてんかん性陰性ミオクローヌスが観察された．この現象は脳波では右中心部-頭頂部-中側頭部からの棘徐波に一致して出現し，同時に記録した左三角筋の筋電図では筋放電の消失を認める（矢印）．較正標は1秒，50 μVを示す．

に下肢が瞬間的に屈曲する．なお，臥位ではENMはわからない．ENMは一側優位に出現することが多い．

3）運動障害などの精神神経症状

ENMがみられる側の手先の不器用さや，動作緩慢が出現する．これは書字や食事などの細かい動作をさせてみるとわかりやすい．これはENMが頻発することに直接関連した現象とも考えられるが，外見的にENMが頻発していないときでも，一側上肢の運動障害が持続する症例も認められる．

NSENMの時期には知的活動の低下，意欲の低下を認めることが多い．過動性行動異常が著明になることもある．

4）経　過

NSENMは寛解，再燃を繰り返すことが多い

が，年齢依存性で，15歳ごろまでには寛解する[8]．上記の運動障害，知的退行，行動異常は寛解期には軽減ないし消失するが，完全には元に戻らない場合もある[8]．

b. 脳 波 像

1) 覚醒時脳波(図2左)

焦点性棘徐波が認められ，二次性全般化を示す．焦点性棘徐波は多焦点性であるが，中心・側頭部からの発射が多く，かつENMが一側優位の場合にはその反対側の中心・側頭部に多くみられ，その部に一過性に徐波の増加を認める．

2) 睡眠時脳波(図2右)

睡眠時にCSWSをみるが，左右差を認めることや，焦点性発射の出現も多く，広汎性棘徐波のspike-wave indexは典型的なECSWSほど高くない[9]．

5. 診断と鑑別診断

a. 診　　断

ENMの頻発を特徴とする発作像と，睡眠時脳波のCSWSが診断に必須である．更に，運動障害，知的退行，行動異常などの精神神経症状を伴えば診断が確実となる．

b. 鑑別診断

1) NSENM，atypical benign partial epilepsy of childhood，ECSWSの関連

1982年，AicardiとChevrieはローランドてんかんの中に，経過中に非定型欠神，失立発作，脱力発作を頻発し，脳波では睡眠中に広汎性発射が頻発する症例群が存在することを報告した．これらの症例では知的退行などの精神神経症状は伴わないとし，良性部分てんかんの亜型と位置付け，atypical benign partial epilepsy of childhood(ABPE)と称した[10]．ここで記載されている脱力発作はENMではないかと考えられる．

ABPEとNSENMの相違点は，ABPEでは非定型欠神やENMの頻発時に精神神経症状を伴わず，知的退行も認められないが，NSENMでは運動障害，知的退行，行動異常などの精神神経症状を認めることが多い点である．また，脳波ではNSENMは多焦点性発射を示すが，ABPEではローランド発射が認められる．

しかし，両者には類似点が多く，ある程度重複していると考えられる．まず，ENMが頻発することと脳波のCSWSの存在は共通している．また，NSENMでは多焦点性発射であるが，多くの場合中心・側頭部からの発射が最も強い点はABPEに似ている．また，NSENMでも睡眠中の部分運動発作がよく認められる点はABPEおよび良性部分てんかんとの関連性を示唆する所見である．

一方，ECSWSは全般発作と部分発作を併有し，脳波にCSWSを認めるてんかん症候群である．ILAEのてんかん分類では徐波睡眠時のspike-wave indexについては規定されていないが，Tassinariらによると，典型的な症例ではこれが85％を超えるという[4]．ECSWSでは高率に知的退行を伴う点が特徴的である．ECSWSは最も早く提唱された概念で，その中にNSENMやABPEなども含まれていたと考えられる．その後関連の病型として，NSENMやABPEが明らかにされてきたため，現在では，ECSWSはCSWSを示すてんかん症候群の中で最もCSWSが典型的なものと位置付けるのが適当と思われる．

以上より，ABPE，NSENMおよびECSWSの3者は連続性をもった年齢依存性の病態と考えられる．3者に共通するのは脳波のCSWSであるが，著者らの検討では，spike-wave indexはECSWSで最も高く，NSENM，ABPEの順に低下していた[9]．このことより，ECSWS，NSENM，ABPE，更に良性部分てんかんは一連のスペクトラム上に位置付けられると考えられる[3,11]．

2) 局在関連性てんかんの経過中に一過性に非けいれん性てんかん重積状態を示す症例

これらの症例が非けいれん性てんかん重積状態に陥ると，非定型欠神のみでなく，しばしば短い脱力発作が頻発し，発作像はNSENMとよく似ているが，睡眠時脳波ではCSWSのパターンはない[12]．また，脱力発作ではNSENMのENMのような左右差が乏しく，短い全身の脱力を認めることが多い[12]．

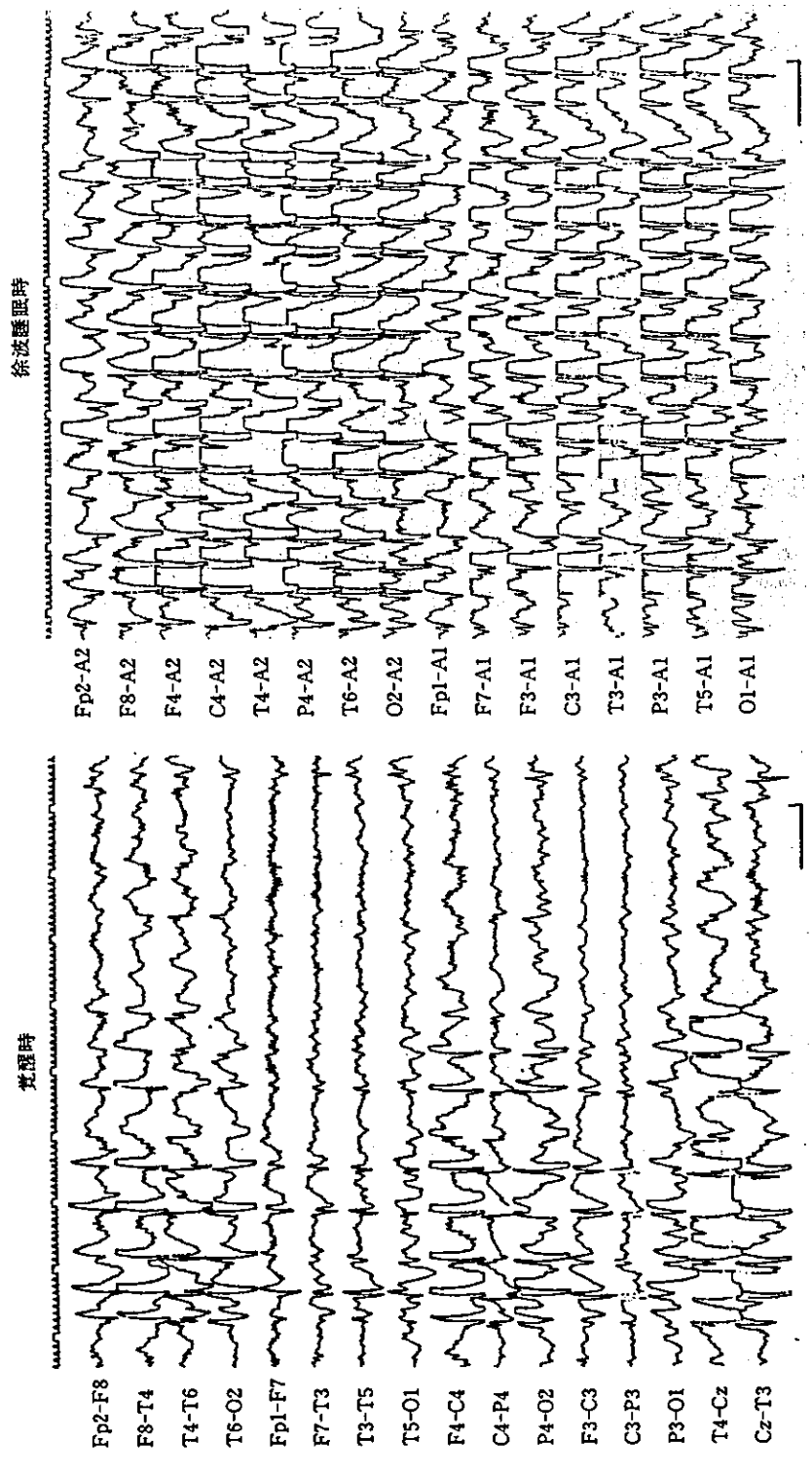

図2 NSENMの脳波

図左の覚醒時には右半球から頻回に棘徐波を認め，同部位に徐波が増加している．図右の徐波睡眠時には持続性棘徐波(CSWS)を認めるが，右半球に棘徐波が強く，左右差がみられる．
較正標は1秒，50 μVを示す．

6. 治療と予後

a. 治　療

ECSWSの治療に準ずる．すなわち，バルプロ酸の大量療法[13]，バルプロ酸とエトスクシミドの併用，ジアゼパムの短期大量療法(ジアゼパム10-20mg/日を就寝前に連日約2週間投与)，合成ACTH療法，ステロイド療法などがある．なお，脱力発作などの小型発作がカルバマゼピンなどの抗てんかん薬により誘発されるという報告[14]があり，注意を要する．特に抗てんかん薬増量中にNSENMが発症したときには，いったんその抗てんかん薬を減量してみることも必要である．

b. 予　後

発作予後はECSWS，ABPEと同じく，最終的には良好で，思春期が終わるまでには寛解する．一方，知能予後に関しては，知的活動はNSENMの時期に低下し，NSENMが寛解すると元のレベルに戻る症例と，再燃と寛解を繰り返すうちに次第に低下し，知的退行を残す症例がある．また，運動障害は基本的にはNSENMが寛解すると改善し，永続的な障害は残らないが，多小脳回を基盤に発症する症例では，再燃と再発を繰り返すうちに次第に片麻痺が増強する症例が認められる[15]．

文　献

1) 大田原俊輔ほか：小児期におけるnon-convulsive status epilepticusの特異型に関する検討．厚生省神経疾患研究委託費「てんかんの成因と治療に関する研究」昭和57年度研究報告書，p67-76，1983.
2) 大塚頌子ほか：小児期におけるnon-convulsive status epilepticusの特異型に関する研究．てんかん研究　1：107-115，1983.
3) 大田原俊輔：ESES症候群．医学のあゆみ　183：96-102，1997.
4) Tassinari CA, et al: Epilepsy with continuous spikes and waves during slow sleep-otherwise described as ESES(epilepsy with electrical status epilepticus during slow sleep). In: Epileptic Syndromes in Infancy, Childhood and Adolescence, 2nd ed(ed by Roger J, et al), p245-256, John Libbey, London, 1992.
5) Guerrini R, et al: Epileptic negative myoclonus. Neurology　43: 1078-1083, 1993.
6) Tassinari CA, et al: Epileptic negative myoclonus. In: Negative Motor Phenomena(ed by Fahn S, et al), p181-197, Lippincott-Raven, Philadelphia, 1995.
7) Commission in Classification and Terminology of ILAE: Proposal of revised classification of epilepsies and epileptic syndromes. Epilepsia　30: 389-399, 1989.
8) 西林尚祐：徐波睡眠期に持続性棘徐波を示すてんかんの縦断的研究．てんかん研究　14：1-11，1996.
9) 小林勝弘ほか：てんかんと睡眠—徐波睡眠中連続的棘徐波を示す非痙攣性てんかん重積状態を中心に—．脳と発達　22：136-142，1990.
10) Aicardi J, Chevrie JJ: Atypical benign partial epilepsy of childhood. Dev Med Child Neurol　24: 281-292, 1982.
11) Doose H, Baier WK: Benign partial epilepsis and related conditions: multifactorial pathogenesis with hereditary impairment of brain maturation. Eur J Pediatr　149: 152-158, 1989.
12) Ohtsuka Y, et al: Nonconvulsive status epilepticus in childhood localization-related epilepsy. Epilepsia　40: 1003-1010, 1999.
13) Ohtsuka Y, et al: Treatment of intractable childhood epilepsy with high-dose valproate. Epilepsia　33: 158-164, 1992.
14) Shields WD, Saslow E: Myoclonic, atonic and absence seizures following institution of carbamazepine therapy in children. Neurology　33: 1487-1489, 1983.
15) Ohtsuka Y, et al: Childhood-onset epilepsy associated with polymicrogyria. Brain Dev, 2002. (in press)

ELSEVIER

Brain & Development 24 (2002) 758–765

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

www.elsevier.com/locate/braindev

Original article

Childhood-onset epilepsy associated with polymicrogyria

Yoko Ohtsuka[a,*], Akio Tanaka[b], Katsuhiro Kobayashi[a], Hodaka Ohta[a], Kiyoko Abiru[a], Kohsuke Nakano[a], Eiji Oka[a]

[a]*Department of Child Neurology, Okayama University Graduate School of Medicine and Dentistry, 2-5-1, Shikatacho, Okayama, Japan*
[b]*Department of Radiology, Okayama University Graduate School of Medicine and Dentistry, 2-5-1, Shikatacho, Okayama, Japan*

Received 12 March 2002; received in revised form 11 June 2002; accepted 12 June 2002

Abstract

To study the electroclinical characteristics of patients with childhood-onset epilepsy who showed polymicrogyria (PMG) on MRI, we classified 15 patients according to the location of PMG on MRI. The composition of the subjects was as follows: four patients with PMG in both hemispheres; three with localized PMG in one hemisphere associated with other lesions such as porencephaly; and eight with only localized PMG in one hemisphere. We investigated the electroclinical characteristics of the epileptic syndromes associated with these different types of PMG. Four patients suffered from infantile spasms during their clinical course. Five patients suffered from epilepsy with electrical status epilepticus during slow sleep (ESES) and ESES-related epilepsy. The other six patients had only localization-related epilepsy throughout their clinical course. Patients with PMG in both hemispheres, and localized PMG in one hemisphere associated with other lesions tended to have early-onset intractable seizures, especially infantile spasms. On the other hand, patients with only localized PMG in one hemisphere had ESES and ESES-related epilepsy or localization-related epilepsy, and their seizure prognosis was relatively favorable. These findings are useful in predicting the outcome of patients with PMG.

Keywords: Polymicrogyria; Cortical malformation; Infantile spasms; Epileptic spasms; Epilepsy with continuous spike-waves during slow wave sleep; Prognosis

1. Introduction

It is well known that cortical malformations are often associated with epilepsy. It is thought that most patients with cortical malformations suffer from intractable seizures. Although cortical malformations such as focal cortical dysplasia are believed to be intrinsically epileptogenic and to cause intractable seizures [1], it has been reported that some patients with multilobar polymicrogyria (PMG) suffer from epilepsy with continuous spike-waves during slow wave sleep (known as epilepsy with electrical status epilepticus during slow sleep or ESES) [2] and show a good long-term seizure prognosis [3–5]. It is likely that different types of cortical malformations have different clinical manifestations. When one investigates the clinical and electroencephalographic (EEG) features of patients with epilepsy associated with cortical malformations, it is important to take into account the specific type of cortical malformation, and recent advances in MRI, such as high-definition MRI, have allowed the physician to identify cortical malformations more precisely and more readily [6]. In this study, we tried to clarify the electroclinical characteristics of epileptic syndromes in an unselected series of patients with PMG as diagnosed by MRI.

2. Subjects and methods

Forty-one patients with epilepsy and cortical malformations as demonstrated by MRI were seen at the Okayama University Hospital between 1991 and 2000. From these, we selected 15 patients (11 males and four females) with PMG for inclusion in this study. The age of the patients at first visit ranged from 2 months to 11 years, 3 months. Age at last follow-up ranged from 1 year, 10 months to 35 years, 5 months.

MRI was performed using a Magnetom H15 unit (1.5 T; Siemens Medical Systems, Germany). T1- and T2-weighted images were obtained. Three-dimensional magnetization-prepared rapid gradient echo (3D MP-RAGE) acquisition with thin partition size (1.4 mm) and evaluation in three planes (axial, coronal and sagittal) were also performed. For detecting PMG, we carefully evaluated cortical patterns and irregularities of the gray–white matter junction [6].

* Corresponding author. Tel.: +81-86-235-7372; fax: +81-86-235-7377.
E-mail address: ohtsuka@md.okayama-u.ac.jp (Y. Ohtsuka).

0387-7604/02/$ - see front matter
PII: S0387-7604(02)00099-2

Because the most specific feature of PMG is irregularities of the gray and white matter junction, we paid particular attention to findings in these areas. To detect irregularities in these areas, we often had to perform evaluation in three planes by means of 3D MP-RAGE. In addition, we carefully checked for abnormally shallow sulci and abnormal patterns of gyri. We included in this study patients whose MRI clearly showed definite features of PMG.

We classified the patients according to the location of PMG on MRI: four patients with PMG in both hemispheres (cases 1–4 in Table 1); three with localized PMG in one hemisphere associated with other lesions such as porencephaly (cases 5–7 in Table 1) and eight with only localized PMG in one hemisphere (cases 8–15 in Table 2). We refer to diffuse or bilateral PMG as type B, unilateral localized PMG associated with other lesions as type UPA and only unilateral localized PMG as type U. We investigated the electroclinical characteristics of the epileptic syndromes associated with these different types of PMG.

We basically classified epileptic syndromes and seizures according to the International League Against Epilepsy (ILAE) classification [7]. In addition, we selected patients whose EEGs showed continuous spike-waves during slow wave sleep (CSWS). These patients shared clinical and EEG characteristics with ESES, but some of them did not completely fit into the pattern of typical ESES [8,9]. We tentatively combined these typical and atypical ESES cases and called them ESES and ESES-related epilepsy. All-night EEGs were recorded in all patients with ESES and ESES-related epilepsy except one (case 10 in Table 2) who underwent only long records including slow wave sleep. The spike-wave index was measured in all patients who underwent all-night EEGs. The spike-wave index was defined as the percentage of time during which diffuse spike-waves occurred, including somewhat asymmetrical diffuse spike-waves but excluding unilateral or focal spike-waves, in the total time of sleep.

3. Results

Age at onset of seizures and the electroclinical characteristics of the various epileptic syndromes are summarized in Tables 1 and 2. Age at onset of seizures ranged from 1 day of life to 16 years, 6 months of age. Seizures started at less than 1 year of age in all type B and type UPA patients except one type B patient (Table 1). All eight type U patients began to have seizures at the age of 1 year or more (Table 2).

Regarding the epileptic syndromes, four patients (two type B patients and two type UPA patients) suffered from infantile spasms during their clinical courses. Five patients (one type B, one type UPA and three type U patients) suffered from ESES and ESES-related epilepsy. The other six patients (one type B patient and five type U patients) had only localization-related epilepsy throughout their clinical courses.

3.1. Four patients with infantile spasms

In three of the four patients with infantile spasms, seizures started in early infancy, ranging from 1 day of life to 2 months of age. In one patient, infantile spasms were preceded by localization-related epilepsy and, in another, by Ohtahara syndrome. Spasms in clusters persisted until the last follow-up in three patients (ages at last follow-up: 1 year, 10 months; 6 years, 2 months and 13 years, 5 months of age). The remaining patient had tonic seizures and isolated spasms at last follow-up. Of the three patients with spasms in clusters at last follow-up, two patients also had partial seizures at last follow-up.

3.2. Five patients with ESES and ESES-related epilepsy

During the appearance of CSWS, we observed epileptic negative myoclonus in three patients, one of whom showed atypical absences as well. Complex partial seizures, secondarily generalized seizures and atypical absences were observed in another. The remaining patient had only one focal motor seizure.

Before the appearance of CSWS, mental and motor activities were considered normal in case 15. Cases 9 and 10 showed mild mental retardation and mild hemiparesis. Case 7 showed moderate mental retardation associated with quadriplegia. Case 3 had severe mental and motor disturbances (quadriplegia). During the appearance of CSWS, two patients (cases 9 and 10) showed aggravation in hemiplegia. In case 15, slight hemiparesis was noticed for the first time during the appearance of CSWS. All patients showed mental deterioration except one who was severely retarded to begin with.

Before and/or after the appearance of CSWS, the interictal EEGs of all patients showed multiple foci, sometimes associated with diffuse spike-waves, mainly during sleep. The laterality of the main focus correlated with the PMG in three type U cases and one type UPA case.

CSWS began to appear before 4 years of age in three patients whose EEGs were recorded before the appearance of this EEG finding. It disappeared between 7 and 15 years of age in three long-term follow-up patients. Although this EEG finding is similar to typical CSWS, it was almost always asymmetrical and was sometimes observed only unilaterally. Moreover, spike-wave indices in all-night sleep recordings were not markedly high in some patients. Spike-wave indices in all-night EEGs were 87% in case 9, 80% in case 15 and 46% in case 3. In case 7, the spike-wave index during the first slow wave sleep reached 78%, but thereafter spike-waves became more asymmetrical and often appeared only unilaterally.

3.3. Seizure prognosis

Seizure prognosis is shown in Tables 1 and 2. Freedom from seizures was defined as being free from seizures for at least 1 year at last follow-up. Among six patients free from

Table 1
Summary of clinical findings of type B and UPA patients[a]

Case no. (gender)	Location of PMG and other lesions	Onset of seizures	Seizure type	EEG findings	Type of epilepsy	Age at last visit	Seizures at last follow-up	Mental dis./Motor dis. at last follow-up
1 (male)	Diffuse bilateral	8 m	Spasms (clusters and isolated), tonic seizure, myoclonic seizure	Hypsarrhythmia → diffuse slow spike-waves	IS (8 m–1 y) → LGS (1 y–)	6 y 2 m	Daily	Severe/severe
2 (male)	Bilateral F–T–Perisylvian	2 m	Spasms (clusters and isolated), tonic seizure, complex partial seizure	Multifocal spikes	IS	1 y 10 m	Daily	Severe/severe
3 (male)	Bilateral F–P–T	5 y 6 m	Focal motor seizure	Bilateral C-mT spikes and diffuse spike-waves → CSWS	ESES and ESES-related epilepsy	8 y 4 m	(–)	Severe/severe
4 (male)	R. Perisylvian (PMG and schizencephaly), L. F–P (PMG)	8 m	Complex partial seizure, secondarily generalized seizure	Multifocal spikes, mainly R. aT	LRE	28 y 8 m	Yearly	Slight/slight
5 (male)	R. P-O (PMG), L. P-O (periventricular nodular heterotopia and porencephaly)	2 m	Spasms in clusters, tonic seizure, focal motor seizure	Hypsarrhythmia → multifocal spikes	LRE → IS (5–7 m) → SGE (3 y–)	20 y 5 m	Daily	Severe/severe
6 (male)	L. F (PMG), dilatation of L. lateral ventricle, hyperintensity in L. thalamus on T2-weighted image	1 day	Spasms (clusters and isolated), focal motor seizure, complex partial seizure	Asymmetric S-B → multifocal spikes	OS (–4 m) → IS	13 y 5 m	Daily	Severe/severe
7 (female)	R. P (PMG), PVL, porencephaly, calcification	7 m	Complex partial seizure, secondarily generalized seizure, atypical absence	Multifocal spikes → CSWS	LRE (7 m–3 y 3 m) → ESES and ESES-related epilepsy	6 y 9 m	Yearly	Moderate/moderate

[a] PMG, polymicrogyria; R, right; L, left; F, frontal; T, temporal; P, parietal; O, occipital; C, central; aT, anterior temporal; mT, mid-temporal; PVL, periventricular leukomalacia; IS, infantile spasms; S-B, suppression-bursts; CSWS, continuous spike-waves during slow wave sleep; LGS, Lennox–Gastaut syndrome; ESES, epilepsy with electrical status epilepticus during slow sleep; LRE, localization-related epilepsy; SGE, symptomatic generalized epilepsy; OS, Ohtahara syndrome; Mental dis., mental disturbance; Motor dis., motor disturbance.

Table 2
Summary of clinical findings of patients with unilateral localized PMG (type U)[a]

Case no. (gender)	Location of PMG	Onset of seizures	Seizure type	EEG findings	Type of epilepsy	Age at last visit	Seizures at last follow-up	Mental dis./Motor dis. at last follow-up
8 (female)	R. F–T–P	3 y 5 m	Complex partial seizure, secondarily generalized seizure	Multifocal spikes and diffuse spike-waves → R. F–T spikes	LRE	14 y 7 m	Monthly	Moderate/slight
9 (male)	R. F–T–P	6 y 8 m	Epileptic negative myoclonus, focal motor seizure	CSWS → R. C-mT spikes	ESES and ESES-related epilepsy	22 y 6 m	(–)	Moderate/slight
10 (male)	L. F–T–P	2 y 3 m	Epileptic negative myoclonus, atypical absence	CSWS → R. mT spikes	ESES and ESES-related epilepsy	24 y 9 m	(–)	Moderate/slight
11 (male)	R. F–P–Perisylvian	12 y 11 m	Secondarily generalized seizure	R.F spikes → no spikes	LRE	31 y 3 m	(–)	Moderate/slight
12 (male)	R. F–P–Perisylvian	16 y 6 m	Complex partial seizure, secondarily generalized seizure	Multifocal spikes, mainly, R. C–P	LRE	18 y 4 m	Monthly	Severe/slight
13 (female)	L. Perisylvian-C	5 y 9 m	Simple partial seizure	Multifocal spikes → L. F–C spikes → no spikes	LRE	35 y 5 m	(–)	Borderline/slight
14 (male)	R. Perisylvian	1 y 0 m	Complex partial seizure, secondarily generalized seizure	R. F spikes	LRE	24 y 10 m	Yearly	Moderate/slight
15 (female)	L. F	1 y 10 m	Epileptic negative myoclonus, focal motor seizure, secondarily generalized seizure	L. F spikes → CSWS → L. C-mT spikes	ESES and ESES-related epilepsy	13 y 2 m	(–)	Slight/slight

[a] PMG, polymicrogyria; R, right; L, left; F, frontal; T, temporal; P, parietal; C, central; mT, mid-temporal; CSWS, continuous spike-waves during slow wave sleep; ESES, epilepsy with electrical status epilepticus during slow sleep; LRE, localization-related epilepsy; Mental dis., mental disturbance; Motor dis., motor disturbance.

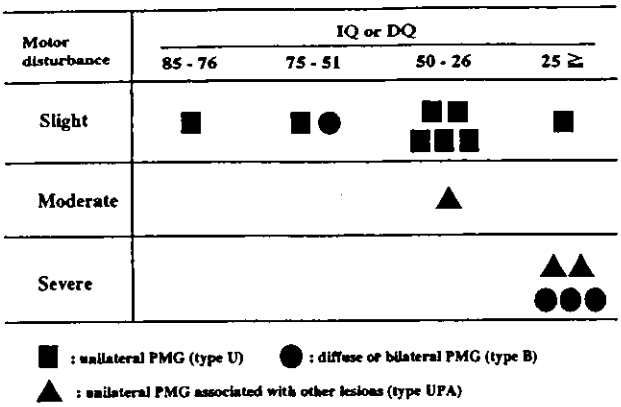

Fig. 1. Developmental outcome. Closed quadrangles and closed triangles indicate type U patients and type UPA patients, respectively. Closed circles indicate type B patients. Patients with slight motor disturbance can walk and deal with daily activity without help in spite of hemiparesis. A patient with moderate motor disturbance can sit and play with toys. Patients with severe motor disturbance cannot perform any normal functions.

seizures, five were type U and the remaining patient was type B. Of these six, four suffered from ESES and ESES-related epilepsy. In contrast, all four patients having daily seizures at last follow-up suffered from infantile spasms. Of these, two were type B and the remaining two were type UPA.

3.4. Developmental outcome

Developmental outcomes are shown in Tables 1 and 2, and Fig. 1. Four patients with infantile spasms showed severe mental and motor disturbances at last follow-up. On the other hand, patients with ESES and ESES-related epilepsy and localization-related epilepsy showed relatively favorable outcomes.

Three of the four type B patients and two of the three type UPA patients showed severe mental and severe motor disturbance at last follow-up. In contrast, seven of the eight type U patients had borderline mentality or slight to moderate mental retardation, and all eight had only slight motor disturbance (Fig. 1).

3.5. PMG localized in one hemisphere and the characteristics of the related epilepsy

The eight type U patients consisted of three with ESES and ESES-related epilepsy and five with localization-related epilepsy. As shown in Table 2, the location and distribution of PMG were similar in patients with ESES and ESES-related epilepsy and those with localization-related epilepsy. In addition, we found that other clinical manifestations, such as the age at onset, seizure prognosis and developmental outcome, were not related to the location and distribution of PMG.

4. Representative cases

4.1. Case 2

This patient is a 1-year-10-month-old boy. He was born after 41 weeks of an uneventful pregnancy. At 2 months of age, he began to have complex partial seizures in which he turned his head to the right or the left while turning his eyes to the same side, associated with a generalized tonic posture. These seizures lasted for several seconds to 1–2 min. At the age of 3 months, his seizure frequency increased to 10–20 times per day and he was admitted to our hospital. While in hospital, he showed two types of seizures: complex partial seizures and spasms in clusters. He sometimes showed combined seizures in which both partial seizures and spasms in clusters occurred successively in one seizure. His interictal EEGs showed multifocal spikes. The ictal EEGs of the complex partial seizures showed bifrontal theta activity, and those of the spasms in clusters showed desynchronization. He continued to have the same types of seizures until the last follow-up. His mental and motor development was severely retarded. His MRI revealed bilateral frontal–temporal–perisylvian PMG (Fig. 2).

4.2. Case 6

This patient is a 13-year-5-month-old boy. He was born after 38 weeks of an eventful pregnancy. However, he began

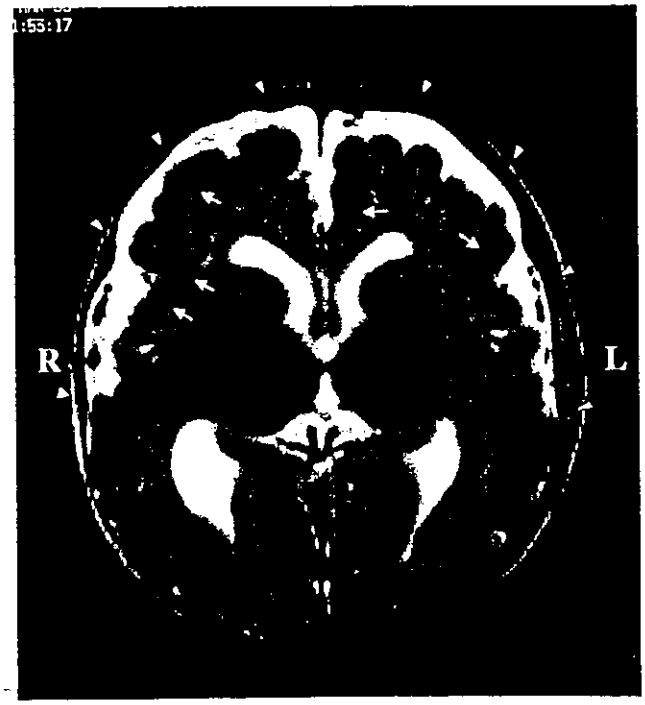

Fig. 2. An axial MRI [T2-weighted image: TR = 3100 ms, TE = 95 ms] of case 2 shows the irregularities of the gray–white matter junction (arrows) in the bilateral frontal–temporal–perisylvian regions at 3 months of age. The irregularities of the gray–white matter junction are seen more clearly in the right side. Note abnormal patterns of the gyri (arrowheads). The bilateral occipital regions look relatively normal.

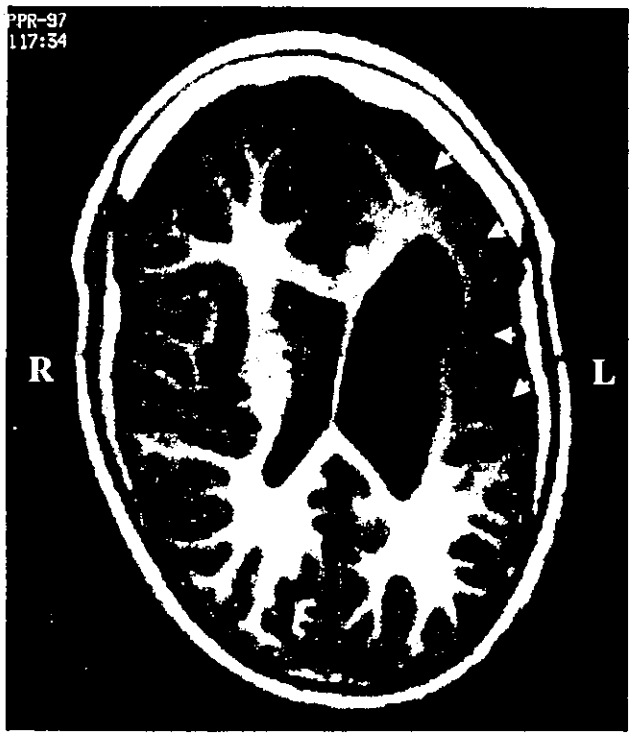

Fig. 3. An axial MRI (3D MP-RAGE) [TR = 10 ms, TE = 4 ms, TI = 300 ms, flip angle = 10°] of case 6 reveals irregularly shallow sulci and abnormal gyral patterns in the left frontal region (arrows) with marked dilatation of the left lateral ventricle at 10 years of age. In addition, a high signal intensity is observed in the left thalamus on a T2-weighted image (not seen in this image).

to have spasms on the first day of life. In addition, focal motor seizures involving the face and limbs appeared at 3 weeks of age. The interictal EEGs showed suppression-burst patterns both during wakefulness and sleep. He was diagnosed as suffering from Ohtahara syndrome. At 6 months of age, his interictal EEGs changed to hypsarrhythmia. He continued to have spasms in clusters and also various types of partial seizures: clonic seizures involving the eyelids, lips, and upper and lower extremities, sometimes associated with asymmetrical tonic posture. Simultaneous video-EEG–electromyography (EMG) recordings revealed that spasms in clusters and partial seizures occurred independently, and also that both types of seizures sometimes appeared successively in one seizure. At present, he is severely handicapped and continues to have spasms in clusters, isolated spasms, partial seizures and combined seizures of spasms in clusters and partial seizures. His MRI showed PMG in the left frontal region, associated with left-side-dominant severe cortical atrophy and hyperintensity in the left thalamus on T2-weighted images (Fig. 3).

4.3. Case 9

This patient is a 22-year-6-month-old man. He was born after 40 weeks of an uneventful pregnancy. He showed psychomotor retardation and left hemiparesis during infancy. At 6 years, 8 months of age, he began to have frequent brief atonic seizures in which his upper body fell forward or his left arm dropped without obvious loss of consciousness. He also had two partial seizures involving the left hand, lasting for less than 1 min during sleep. The frequency of the brief atonic seizures increased up to once every several seconds to several minutes. Intravenous injection of diazepam suppressed these seizures. However, five or six clusters of frequent but brief atonic seizures recurred until he was 15 years, 10 months old. These clusters of brief atonic seizures lasted for several weeks to several months. Simultaneous EEG–EMG recordings revealed that these seizures were epileptic negative myoclonus [10,11] involving the left arm associated with right central spike-wave discharges on EEG. During these periods his hemiparesis and his mental activity worsened. His mental functions partly recovered after these periods, but his IQ gradually worsened from 51 to 29 during the 6 years after onset. His motor disturbances also followed a similar course to his mental activity. During these periods, interictal EEGs showed CSWS which were almost always asymmetrical and were sometimes observed only unilaterally. The spike-wave index of the all-night EEG reached 87%. While he was free from epileptic negative myoclonus, his EEGs showed focal spike-waves, mainly in the right central-mid-temporal regions, sometimes associated with diffuse spike-waves mainly during sleep. MRI revealed

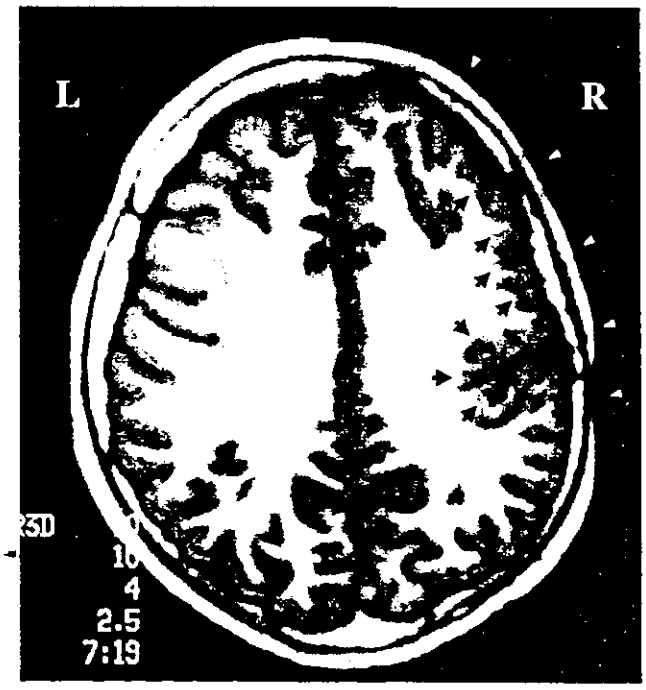

Fig. 4. An axial MRI image (3D MP-RAGE) [TR = 10 ms, TE = 4 ms, TI = 300 ms, flip angle = 10°] of case 9 reveals multilobar PMG in the right frontal–parietal–temporal regions at 16 years, 4 months of age. The arrows indicate the irregularities of the gray–white matter junction. The arrowheads indicate irregularly shallow and abnormal gyral patterns.

multilobar PMG involving the right frontal–parietal–temporal regions (Fig. 4).

5. Discussion

This study revealed that patients with diffuse or bilateral PMG (type B) and unilateral localized PMG associated with other lesions (type UPA) tended to have early-onset intractable seizures, especially infantile spasms. On the other hand, patients with unilateral localized PMG (type U) had ESES and ESES-related epilepsy or localization-related epilepsy, and their clinical course and prognosis were relatively favorable. Thus, the developmental outcome was closely related to epileptic syndrome and also the extent of cortical malformation, since it was less favorable in types B and UPA than in type U.

There are an increasing number of reports on infantile spasms or epileptic spasms associated with cortical malformations [12]. In these reports, the cortical malformations associated with these seizure types were lissencephaly, including diffuse pachygyria, [13,14], band heterotopia [14], hemimegalencephaly [15,16], bilateral perisylvian PMG [17], bilateral occipital PMG [13] and localized cortical malformation [18–25]. In most of these cases, the localized cortical malformations are focal cortical dysplasia, although detailed data are not available in some cases. Thus, in these previously reported cases of PMG, infantile spasms or epileptic spasms were mostly seen in diffuse or bilateral PMG cases. Regarding bilateral PMG, bilateral perisylvian PMG is a well-known condition, and bilateral parasagittal parietooccipital PMG [26] and bilateral frontal PMG [27] have recently been reported on. Although it has been reported that some patients with bilateral perisylvian PMG suffer from infantile spasms [17], no cases of infantile spasms have been reported among other types of bilateral PMG, except for one patient with bilateral occipital PMG [13]. In contrast, one of three patients in our study with bilateral PMG suffered from infantile spasms. Therefore, we believe that recent advances in neuroimaging will enable us to diagnose more and more cases of infantile spasms associated with bilateral PMG in the future. Although two of three type UPA cases in our study had infantile spasms, they also had other severe brain damage or malformations in addition to PMG. Therefore, the combination of unilateral localized PMG and other forms of brain damage, or only the latter might have caused the infantile spasms.

In most reported cases with cortical malformations, spasms in clusters start before 1 year of age, sometimes as early as in the neonatal period [15,16,23,24]. During their clinical course, these patients often have partial seizures as well as spasms in clusters. Partial seizures tend to occur during the period of appearance of spasms in clusters, especially as 'combined seizures' or 'simultaneous seizures' [12,14,20–22]. The interictal EEGs of these patients show not only hypsarrhythmia but also other EEG patterns, such as suppression-burst patterns [23,24] and multifocal spikes [14]. Our cases of PMG associated with infantile spasms showed the same clinical and EEG characteristics as these previously mentioned reported cases of cortical malformations.

Recently, Guerrini et al. [3,4] suggested a close relationship between multilobar PMG and ESES. ESES is classified as "epilepsies and syndromes undetermined whether focal or generalized" in the ILAE classification [7]. Therefore, typical cases of ESES have both focal and generalized seizures together or in succession (e.g. partial seizures plus absences), and have both focal and generalized EEG seizure discharges [7]. According to this definition, some of our cases had atypical clinical features. Our case 3 had only one partial motor seizure. As for EEG findings, typical cases of ESES show very high spike-wave indices (85% or higher) [2]. Among our patients, only one reached this high level. Although the EEG findings observed in our patients were similar to typical CSWS, they were almost always asymmetrical and were sometimes observed only unilaterally. Diffuse spike-waves became more asymmetrical and sometimes became exclusively unilateral as sleep went on during all-night sleep records. During the clinical course, the same patient showed only diffuse continuous spike-waves during slow wave sleep in some occasions or only unilateral continuous spike-waves on other occasions. These phenomena are rarely seen in typical cryptogenic cases of ESES [2].

Guerrini et al. [3,4] also reported that patients with multilobar PMG and ESES did not show definite cognitive deterioration after ESES compared with earlier evaluations. However, all but one of our patients showed mental deterioration during the period of CSWS. All of our patients had motor disturbances, including hemiplegia in three patients. These motor disturbances worsened transiently during the period of appearance of CSWS. As CSWS recurred several times, the mental and motor disturbances showed gradually increased severity in some cases. This type of motor deterioration is rarely observed in typical cryptogenic ESES patients [2]. On the other hand, our cases showed favorable seizure prognoses, which is compatible with the data reported by other researchers [2–4].

In addition to cases of ESES and ESES-related epilepsy, type U patients who had localization-related epilepsy also showed relatively favorable seizure prognoses and developmental outcomes. Compared with focal cortical dysplasia, which itself is intrinsically epileptogenic [1], the entire area of PMG may not be epileptogenic [28]. Thus, large PMG might not necessarily indicate a large epileptogenic area or strong epileptogenicity.

Although recent advances in MRI have allowed the physician to identify cortical malformations more precisely and more readily [6], there is still a significant number of patients with cortical malformations whose exact nature cannot be clarified radiologically. Furthermore, the precise extent of PMG is sometimes difficult to determine radiologically. Therefore, we classified our patients roughly into

three groups according to the location of PMG on MRI. In spite of these limitations, this study reveals the electroclinical characteristics of patients with MRI-visible PMG. We believe that these findings will be useful in predicting the outcome of patients with PMG.

Acknowledgements

This study was supported in part by a research grant (7 Shi-1) for Nervous and Mental Disorders from the Ministry of Health and Welfare, Japan.

References

[1] Palmini A, Gambardella A, Andermann F, Dubeau F, da Costa JC, Olivier A, et al. Intrinsic epileptogenicity of human dysplastic cortex as suggested by corticography and surgical results. Ann Neurol 1995;37:476–487.

[2] Tassinari CA, Bureau M, Dravet C, Dalla Bernardina B, Roger J. Epilepsy with continuous spikes and waves during slow sleep – otherwise described as ESES (epilepsy with electrical status epilepticus during slow sleep). In: Roger J, Bureau M, Dravet C, Dreifuss FE, Perret A, Wolf P, editors. Epileptic syndromes in infancy, childhood and adolescence, 2nd ed.. London: John Libbey, 1992. pp. 245–256.

[3] Guerrini R, Parmeggiani A, Bureau M, Dravet C, Genton P, Salas-Puig X, et al. Localized cortical dysplasia: good seizure outcome after sleep-related electrical status epilepticus. In: Guerrini R, Andermann F, Canapicchi R, Roger J, Zifkin BG, Pfanner P, editors. Dysplasias of cerebral cortex and epilepsy, Philadelphia: Lippincott-Raven, 1996. pp. 329–335.

[4] Guerrini R, Genton P, Bureau M, Parmeggiani A, Salas-Puig X, Santucci M, et al. Multilobar polymicrogyria, intractable drop attack seizures, and sleep-related electrical status epilepticus. Neurology 1998;51:504–512.

[5] Caraballo R, Cersósimo R, Fejerman N. A particular type of epilepsy in children with congenital hemiparesis associated with unilateral polymicrogyria. Epilepsia 1999;40:865–871.

[6] Barkovich AJ. Magnetic resonance imaging of lissencephaly, polymicrogyria, schizencephaly, hemimegalencephaly, and band heterotopia. In: Guerrini R, Andermann F, Canapicchi R, Roger J, Zifkin BG, Pfanner P, editors. Dysplasias of cerebral cortex and epilepsy, Philadelphia, PA: Lippincott-Raven, 1996. pp. 115–129.

[7] Commission on Classification and Terminology of the International League Against Epilepsy. Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia 1989;30:389–399.

[8] Ohtsuka Y, Yoshida H, Matsuda M, Terasaki T, Iyoda K, Yamatogi Y, et al. A peculiar type of nonconvulsive status epilepticus in childhood: a clinical and electroencephalographic study. J Jpn Epil Soc 1983;1:107–115.

[9] Kobayashi K, Murakami N, Yoshinaga H, Enoki H, Ohtsuka Y, Ohtahara S. Nonconvulsive status epilepticus with continuous diffuse spike-and-wave discharges during sleep in childhood. Jpn J Psychiatry Neurol 1988;42:509–514.

[10] Guerrini R, Dravet C, Genton P, Bureau M, Roger J, Rubboli G, et al. Epileptic negative myoclonus. Neurology 1993;43:1078–1083.

[11] Tassinari CA, Rubboli G, Parmeggiani L, Valzania F, Plasmati R, Riguzzi P, et al. Epileptic negative myoclonus. In: Fahn S, Hallet M, Lüders HO, Marsden CD, editors. Negative motor phenomena, Advances in Neurology, vol. 67. Philadelphia, PA: Lippincott-Raven, 1995. pp. 181–197.

[12] Kobayashi K, Ohtsuka Y, Ohno S, Ohmori I, Ogino T, Yoshinaga H, et al. Clinical spectrum of epileptic spasms associated with cortical malformation. Neuropediatrics 2001;32:236–244.

[13] Carrazana EJ, Lombroso CT, Mikati M, Helmers S, Holmes GL. Facilitation of infantile spasms by partial seizures. Epilepsia 1993;34:97–109.

[14] Gobbi G, Pini A, Parmeggiani A, Santucci M, Giovanardi Rossi P, Guerrini R. Periodic spasms in cortical dysplasia. In: Guerrini R, Andermann F, Canapicchi R, Roger J, Zifkin BG, Pfanner P, editors. Dysplasias of cerebral cortex and epilepsy, Philadelphia, PA: Lippincott-Raven, 1996. pp. 311–321.

[15] Paladin F, Chiron C, Dulac O, Plouin P, Ponsot G. Electroencephalographic aspects of hemimegalencephaly. Dev Med Child Neurol 1989;31:377–383.

[16] Vigevano F, Bertini E, Boldrini R, Bosman C, Claps D, di Capua M, et al. Hemimegalencephaly and intractable epilepsy: benefits of hemispherectomy. Epilepsia 1989;30:833–843.

[17] Kuzniecky R, Andermann F, Guerrini R. Infantile spasms: an early epileptic manifestation in some patients with the congenital bilateral perisylvian syndrome. J Child Neurol 1994;9:420–423.

[18] Chugani HT, Shields WD, Shewmon DA, Olson DM, Phelps ME, Peacock WJ. Infantile spasms: I. PET identifies focal cortical dysgenesis in cryptogenic cases for surgical treatment. Ann Neurol 1990;27:406–413.

[19] Guerrini R, Dravet C, Raybaud C, Roger J, Bureau M, Battaglia A, et al. Epilepsy and focal gyral anomalies detected by MRI: electroclinico-morphological correlations and follow-up. Dev Med Child Neurol 1992;34:706–718.

[20] Kubota T, Aso K, Negoro T, Okumura A, Natsume J, Takada H, et al. Epileptic spasms preceded by partial seizures with a close temporal association. Epilepsia 1999;40:1572–1579.

[21] Ohtsuka Y, Murashima I, Asano T, Oka E, Ohtahara S. Partial seizures in West syndrome. Epilepsia 1996;37:1060–1067.

[22] Ohtsuka Y. West syndrome and its related epileptic syndromes. Epilepsia 1998;39(Suppl 5):30–37.

[23] Ohtsuka Y, Sato M, Sanada S, Yoshinaga H, Oka E. Suppression-burst patterns in intractable epilepsy with focal cortical dysplasia. Brain Dev 2000;22:135–138.

[24] Pedespan JM, Loiseau H, Vital A, Marchal C, Fontan D, Rougier A. Surgical treatment of an early epileptic encephalopathy with suppression-bursts and focal cortical dysplasia. Epilepsia 1995;36:37–40.

[25] Watanabe K, Negoro T, Aso K, Maeda N, Ohki T, Hayakawa F, et al. Clinical, EEG, and positron emission tomography features of childhood-onset epilepsy with localized cortical dysplasia detected by magnetic resonance imaging. J Epilepsy 1994;7:108–116.

[26] Guerrini R, Dubeau F, Dulac O, Barkovich AJ, Kuzniecky R, Fett C, et al. Bilateral parasagittal parietooccipital polymicrogyria and epilepsy. Ann Neurol 1997;41:65–73.

[27] Guerrini R, Barkovich AJ, Sztriha L, Dobyns WB. Bilateral frontal polymicrogyria: a newly recognized brain malformation syndrome. Neurology 2000;54:909–913.

[28] Sisodiya SM. Surgery for malformations of cortical development causing epilepsy. Brain 2000;123:1075–1091.

ACADEMIC PRESS

Biochemical and Biophysical Research Communications 295 (2002) 17–23

BBRC

www.academicpress.com

Significant correlation of the SCN1A mutations and severe myoclonic epilepsy in infancy☆

Iori Ohmori,[a,b] Mamoru Ouchida,[b,*] Yoko Ohtsuka,[a] Eiji Oka,[a] and Kenji Shimizu[b]

[a] *Department of Child Neurology, Graduate School of Medicine and Dentistry, Okayama University, Shikata-cho 2-5-1, Okayama-shi, Okayama 700-8558, Japan*

[b] *Department of Molecular Genetics, Graduate School of Medicine and Dentistry, Okayama University, Shikata-cho 2-5-1, Okayama-shi, Okayama 700-8558, Japan*

Received 15 May 2002

Abstract

To investigate the possible correlation between genotype and phenotype of epilepsy, we analyzed the voltage-gated sodium channel α1-subunit (SCN1A) gene, β1-subunit (SCN1B) gene, and γ-aminobutyric $acid_A$ receptor γ2-subunit (GABRG2) gene in DNAs from peripheral blood cells of 29 patients with severe myoclonic epilepsy in infancy (SME) and 11 patients with other types of epilepsy. Mutations of the SCN1A gene were detected in 24 of the 29 patients (82.7%) with SME, although none with other types of epilepsy. The mutations included deletion, insertion, missense, and nonsense mutations. We could not find any mutations of the SCN1B and GABRG2 genes in all patients. Our data suggested that the SCN1A mutations were significantly correlated with SME ($p < .0001$). As we could not find SCN1A mutations in their parents, one of critical causes of SME may be de novo mutation of the SCN1A gene occurred in the course of meiosis in the parents.

Keywords: Neuronal voltage-gated sodium channel; SCN1A; SCN1B; GABRG2; Generalized epilepsy with febrile seizures plus; Sever myoclonic epilepsy in infancy

Generalized epilepsy with febrile seizures plus (GEFS+) is a benign childhood-onset epileptic syndrome with autosomal dominant inheritance [1,2]. This syndrome is characterized by febrile seizures that persist beyond 6 years of age and by an appearance of other types of afebrile seizures. In the GEFS+ family members, phenotypic and genetic heterogeneities are seen. GEFS+ includes childhood absence epilepsy, myoclonic-astatic epilepsy (MAE), severe myoclonic epilepsy in infancy (SME) [3], idiopathic generalized epilepsy (IGE), and localization-related epilepsy (LRE). Among these epileptic syndromes, SME is considered to be the most severe phenotype within the GEFS+ spectrum [4]. SME is a malignant epileptic syndrome, while GEFS+ is usually benign. It is very important to elucidate the molecular genetic basis of SME.

Epileptic seizures are induced by abnormal electrical discharges within the brain. Since ion channels are fundamental in membrane potential generation, mutations of genes encoding some types of ion channels may cause epilepsy. Recent molecular genetic studies have revealed that mutations of the voltage-gated sodium channel α1-subunit (SCN1A) gene [5–10], α2-subunit (SCN2A) gene [11], β1-subunit (SCN1B) gene [12], and γ-aminobutyric acid $(GABA)_A$ receptor γ2-subunit (GABRG2) gene [13,14] were detected in patients with GEFS+, while there have been only a few reports of mutation analysis in the patients with SME [15–17].

To find the causing genes of SME and clarify the possible correlation between the genotype and phenotype of epilepsy, we performed mutation analysis of the SCN1A, SCN1B, and GABRG2 genes in the patients with SME and other types of epilepsy.

☆ *Abbreviations:* SME, severe myoclonic epilepsy in infancy; SCN1A, sodium channel α1-subunit; SCN1B, sodium channel β1-subunit; GABRG2, γ-aminobutyric $acid_A$ receptor γ2-subunit; GEFS+, generalized epilepsy with febrile seizures plus; MAE, myoclonic-astatic epilepsy; LRE, localization-related epilepsy; IGE, idiopathic generalized epilepsy; bp, base pair.

* Corresponding author. Fax: +81-86-235-7383.
E-mail address: ouchidam@md.okayama-u.ac.jp (M. Ouchida).

0006-291X/02/$ - see front matter
PII: S0006-291X(02)00617-4

Materials and methods

Patients. Twenty-nine patients with SME and eleven patients with other types of epilepsy including MAE, IGE, and LRE were recruited who were seen at Okayama University Hospital from 1969 to 2001. Epileptic syndromes were classified according to the diagnostic criteria of the International League against Epilepsy classification [18]. Clinical features of patients were presented in Table 1.

DNA preparation. Peripheral blood was acquired from the patients and their parents after written informed consent was obtained from all participants. Genomic DNA was extracted from peripheral blood leukocytes by DNA extract WB Kit (Nippon gene, Tokyo, Japan).

PCR amplification and DNA sequencing. Twenty-six exons of the SCN1A gene, five exons of the SCN1B gene, and nine exons of the GABRG2 gene were amplified with the intronic primers [12,17]. The SCN1A gene was amplified with a pair of primers as follows: exon 1 sense 5′-TCATGGCACAGTTCCTGTATC and antisense 5′-GCAGT AGGCAATTAGCAGCAA, exon 2 sense 5′-TGGGGCACTTTAG AAATTGTG and antisense 5′-TGACAAAGATGCAAAATGAG AG, exon 3 sense 5′-GCAGTTTGGGCTTTTCAATG and antisense 5′-TGAGCATTGTCCTCTTGCTG, exon 4 sense 5′-AGGGCTACG TTTCATTTGTATG and antisense 5′-TGTGCTAAATTGAAATC CAGAG, exon 5 sense 5′-CAGCTCTTCGCACTTTCAGA and antisense 5′-TCAAGCAGAGAAGGATGCTGA, exon 6 sense 5′-AGC GTTGCAAACATTCTTGG and antisense 5′-GGGATATCCAG CCCCTCAAG, exon 7 sense 5′-GACAAATACTTGTGCCTTTGAA TG and antisense 5′-ACATAATCTCATACTTTA TCAAAAACC, exon 8 sense 5′-GAAATGGAGGTGTTGAAAA TGC and antisense 5′-AATCCTTGGCATCACTCTGC, exon 9 sense 5′-AGTACAGGG TGCTATGACCAAC and antisense 5′-TCCTCATACAACCACC TGCTC, exon 10 sense 5′-TCTCCAAAAGCCTTCATTAGG and antisense 5′-TTCTAATTCTCCCCCTCTCTCC, exon 11 sense 5′-TC CTCATTCTTTAATCCCAAGG and antisense 5′-GCCGTTCTGT AGAAACACTGG, exon 12 sense 5′-GTCA GAAATATCTGCCAT CACC and antisense 5′-GAATGCACTATT CCCAACTCAC, exon 13 sense 5′-TGGGCTCTATGTGTGTGTCTG and antisense 5′-GGA

Table 1
Summary of the clinical features of patients

Patients no.	Type of epilepsy	Sex	Age at onset	Family history of onset convulsive disorders
1	SME	M	5 m	Paternal aunt and paternal GF:FS
2	SME	M	7 m	—
3	SME	M	3 m	Father:FS
4	SME	F	4 m	—
5	SME	F	4 m	—
6	SME	F	2 m	Father and paternal:FS
7	SME	F	3 m	Brother:FS
8	SME	M	5 m	Brother:FS
9	SME	M	5 m	—
10	SME	F	6 m	—
11	SME	M	4 m	Paternal uncle:FS
12	SME	M	5 m	Paternal aunt:FS
13	SME	M	7 m	—
14	SME	F	6 m	—
15	SME	F	5 m	Maternal cousin:FS
16	SME	M	6 m	Father and sister:FS
17	SME	F	7 m	—
18	SME	M	7 m	Sister and paternal GF:FS
19	SME	F	4 m	—
20	SME	M	8 m	—
21	SME	F	3 m	Paternal cousin:FS
22	SME	F	5 m	Mother:Ep, sister:FS
23	SME	F	6 m	—
24	SME	F	7 m	—
25	SME	F	4 m	Father and paternal GF:FS
26	SME	F	4 m	Brother:FS
27	SME	F	6 m	Paternal GF and brother:Ep
28	SME	F	6 m	—
29	SME	M	4 m	—
30	MAE	M	8 m	Maternal cousin:FS
31	MAE	M	2 y 10 m	—
32	MAE	F	2 y 9 m	Maternal cousin:FS
33	MAE	M	7 m	—
34	IGE	F	8 m	Father:FS
35	IGE	M	2 y 10 m	Father and paternal cousin:FS
36	IGE	F	1 y 3 m	—
37	IGE	M	5 y 6 m	—
38	IGE	M	1 y 0 m	Father:FS
39	LRE	M	1 y 2 m	—
40	LRE	M	3 y 1 m	—

Note. SME, severe myoclonic epilepsy in infancy; MAE, myoclonic-astatic epilepsy; IGE, idiopathic generalized epilepsy; LRE, localization-related epilepsy; M, male; F, female; m, months; y, years; GF, grandfather; FS, febrile seizure; Ep, epilepsy.

AGCATGAAGGATGGTTG, exon 14 sense 5′-TACTTCGCGTTT CCACAAGG and antisense 5′-GCTATGCAAGAACCCTGATTG, exon 15 sense 5′-ATGAGCCTGA GACGGTTAGG and antisense 5′-ATACATGTGCCATGCTGGTG, exon 16 sense 5′-TGCTGTG GTGTTTCCTTCTC and antisense 5′-TGTATTCATACCTTCCC ACACC, exon 17 sense 5′-AAAAGGGTTAGCACAGACAATG and antisense 5′-ATTGGGCAGATATAATCAAAGC, exon 18 sense 5′-C ACACAGCTGATGAATGTGC and antisense 5′-TG AAGGGCTA CACTTTCTGG, exon 19 sense 5′-TCTGCCCTCCTATTCCAATG and antisense 5′-GCCCTTGTCTTCCAGAAATG, exon 20 sense 5′-AAAAATTACATCCTTTACATCAAACTG and antisense 5′-TT TTGCATGCATAGATTTTCC, exon 21 sense 5′-TGAACCTTGC TTTTACATATCC and antisense 5′-ACCCATCTGGG CTCATA AAC, exon 22 sense 5′-TGTCTTGGTCCAAAATCTGTG and antisense 5′-TTGGTCGTTTATGCTTTATTCG, exon 23 sense 5′-CCC TAAAGGCCAATTTCAGG and antisense 5′-ATTTGGCAGA GAAAACACTCC, exon 24 sense 5′-GAGATTTGGGGGTGTT TGTC and antisense 5′-GGATT GTAATGGGGTGCTTC, exon 25 sense 5′-CAAAAATCAGGGCC AATGAC and antisense 5′-TG ATTGCTGGGATGATCTTG, exon 26(1) sense 5′-AGGACTCTG AACCTTACCTTGG and antisense 5′-CCATGAATCGCTCTTCC ATC, exon 26(2) sense 5′-TGTGGGAA CCCATCTGTTG and antisense 5′-GTTTGCTGACAAGGGGTCAC. PCR was performed in 25 μl reaction volume containing 50 ng of genomic DNA, 20 pmol of each primer, 0.8 mM dNTPs, 1× reaction buffer, 1.5 mM $MgCl_2$, and 0.7 U of AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA, USA). All PCR products were purified with a PCR products pre-sequencing kit (Amersham Biosciences, Little Chalfont, Buckinghamshire, England), reacted with the Big Dye Terminator FS ready-reaction kit (Applied Biosystems), and analyzed on an ABI PRISM3100 sequencer (Applied Biosystems). Mutations we found were confirmed twice by independent PCR amplification and sequencing.

Statistical analysis. Fisher's exact test was used for the statistical analysis to clarify the correlation between the mutations and subgroup of epilepsy.

Results

Mutation analysis

The sodium channels are composed of a pore-forming α-subunit and one or more smaller β-subunit(s). The β-subunit enhances the Na^+ current amplitude and modifies its properties [19]. We analyzed the SCN1A, SCN1B, and GABRG2 genes in DNAs from peripheral blood cells of 29 patients with SME and 11 patients with other types of epilepsy within GEFS+ spectrum. The clinical features of patients are shown in Table 1. Interestingly, mutations of the SCN1A gene were detected in 24 of the 29 patients (82.7%) with SME, while there was no mutation in the patients with other types of epilepsy (Table 2). Various types of mutations including missense and nonsense mutations and frameshift mutation by insertion or deletion were detected. Representative data of missense, nonsense, and frameshift mutations were shown in Fig. 1A. Location of the all mutations on the sodium channel α1-subunit was schematically presented in Fig. 1B, in which the mutations leading the premature termination by nonsense and frameshift mutations are distinctly shown to the mutations including missense mutation and 3-bp deletion. The protein has four homologous repeats (domain 1–4), and a domain and the neighbor one are linked with a loop structure. Each of these domains contains six transmembrane segments (S1–S6). The mutations were mainly located in the intracellular loop 1, loop 2, and S5–S6 pore regions. In Fig. 2, position of the representative missense mutations is indicated on amino acid sequence similarity of sodium channel α-subunit gene family. The amino acids that were substituted by missense mutation were highly conserved in the wide species including Drosophila, not only in these three cases shown in Fig. 2 but also in the others (data not shown). No mutations were detected in the patients' parents we tested (Table 2). The substitution of threonine with alanine at codon 1057 in the patients 1, 6, 15, 21, and their parents seems to be a single nucleotide polymorphism (SNP), because Escayg et al. reported that the rate of alanine allele on this SNP is 0.33 in the normal population [6]. We could not find any mutations of the SCN1B and GABRG2 genes in all patients (data not shown).

High correlation of the SCN1A mutation and SME

When statistical analysis was performed on SME and other types of epilepsy, we found a significant correlation between the SCN1A mutation and SME ($p < .0001$). As regards clinical manifestations, all patients with SME had intractable epileptic seizures. Various degrees of ataxia were observed in all except for patients 8, 13, 17, 23, and 24. All patients had slight to severe mental retardation. Patients 8, 17, 23, and 24 showed less severe mental impairment than the other patients did. We could not find significant correlation between the type of the SCN1A mutations and clinical severity.

Discussion

There have been only a few reports of mutation analysis in the patients with SME. Claes et al. first described seven mutations of the SCN1A gene [15] and Sugawara et al. recently reported 10 mutations of the SCN1A gene in 14 patients with SME [16]. Only one GABRG2 mutation was found in a patient with SME in a family with GEFS+ [17]. The present study is the largest series of comprehensive mutation screening in the patients with SME and other types of epilepsy. By direct sequencing of all coding exons of the SCN1A, SCN1B, and GABRG2 genes, we detected 24 mutations of the SCN1A gene in the 29 patients with SME (82.7%) while none with other types of epilepsy. Our data suggested that the SCN1A mutation was significantly correlated with SME ($p < .0001$), although the number of other types of epilepsy was not so many. Escayg et al.

Table 2
Mutations in the SCN1A gene

Patients no.	Type of epilepsy	Exon	Nucleotide change[a]	Amino acid change[b]	Mutation type	Position	The same mutations in	
							Father	Mother
1	SME	Exon 10	G1502del	R501fsX543	Frameshift	loop1	–	–
		Exon 16	A3169G	T1057A	Polymorphism	loop2	+	–
2	SME	Exon 10	1641insA	K547fsX570	Frameshift	loop1	–	–
3	SME	Exon 11	C1702T	R568X	Nonsense	loop1	–	–
4	SME	Exon 11	C1820del	S607fsX622	Frameshift	loop1	NE	–
5	SME	Exon 12	C2087del	P696fsX703	Frameshift	loop1	–	–
6	SME	Exon 12	C2101T	R701X	Nonsense	loop1	–	–
		Exon 16	A3169G	T1057A	Polymorphism	loop2	–	+
7	SME	Exon 15	C2560T	R854X	Nonsense	DIIS3-S4loop	–	–
8	SME	Exon 15	T2672G	F891C	Missense	DIIS4-S5 loop	NE	–
9	SME	Exon 15	C2761T	R921C	Missense	DIIS5-S6 pore	–	–
10	SME	Exon 15	C2761T	R921C	Missense	DIIS5-S6 pore	–	–
11	SME	Exon 16	C2976del	A992fsX999	Frameshift	DIIS6	–	–
12	SME	Exon 16	A3049T	K1017X	Nonsense	loop2	–	–
13	SME	Exon 16	C3215del	T1072fsX1077	Frameshift	loop2	–	–
14	SME	Exon 19	T3764C	L1255P	Missense	DIIIS2	–	–
15	SME	Exon 19	G3782A	W1261X	Nonsense	DIIIS2	NE	NE
		Exon 16	A3169G	T1057A	Polymorphism	loop2	NE	NE
16	SME	Exon 19	CTT3837-3839del	F1279del	Deletion	DIIIS2-S3 loop	NE	NE
17	SME	Exon 21	A3169G	V1380M	Missense	DIIIS5-S6 pore	NE	NE
18	SME	Exon 22	4256-4260 del(CCACA) ins(ATGTCC)	A1419fsX1433	Frameshift	DIIIS5-S6 pore	NE	NE
19	SME	Exon 22	T4270C	W1424R	Missense	DIIIS5-S6 pore	NE	NE
20	SME	Exon 23	A4319G	Q1440R	Missense	DIIIS5-S6 pore	–	–
21	SME	Exon 26	C4912T	R1638C	Missense	DIVS4	NE	–
		Exon 16	A3169G	T1057A	Polymorphism	loop2	NE	–
22	SME	Exon 26	G4990C	G1664R	Missense	DIVS4-S5 loop	–	–
23	SME	Exon 26	5610-5615 del(AGAGAT) ins(CTAGAGTA)	G1870fsX1871	Frameshift	C-terminal	NE	NE
24	SME	Exon 26	C5696T	T1899I	Missense	C-terminal	NE	NE
25	SME	–	–				NE	NE
26	SME	–	–				NE	NE
27	SME	–	–				NE	NE
28	SME	–	–				NE	NE
29	SME	–	–				NE	NE
30	MAE	–	–				NE	NE
31	MAE	–	–				NE	NE
32	MAE	–	–				NE	NE
33	MAE	–	–				NE	NE
34	I GE	–	–				NE	NE
35	I GE	–	–				NE	NE
36	I GE	–	–				NE	NE
37	I GE	–	–				NE	NE
38	I GE	–	–				NE	NE
39	LRE	–	–				NE	NE
40	LRE	–	–				NE	NE

[a] Nucleic acid numbering started from A of the intiating ATG.
[b] Amino acid numbering started from the intiating ATG codon. del, deletion; ins, insertion; fs, frameshift; X, stop codon; DII, Domain 2; DIII, Domain 3; DIV, Domain 4; NE, not examined; –, negative; +, positive.

reported that the rate of the SCN1A mutation in GEFS+ was 5.6% [6]. In their study, the subjects included mainly juvenile myoclonic epilepsy and childhood absence epilepsy except SME. The prominent clinical feature of SME is the frequent occurrence of convulsions associated with fever, which sometimes develop to status epilepticus. The Japanese authors reported that hot baths could also provoke epileptic seizures [20,21]. These clinical manifestations are very rare among other epileptic syndromes. Since the SCN1A mutations were frequently detected in SME but not in other types of epilepsy, this channel may be related to a dysfunction of the brain neurons exacerbated by high body temperature.

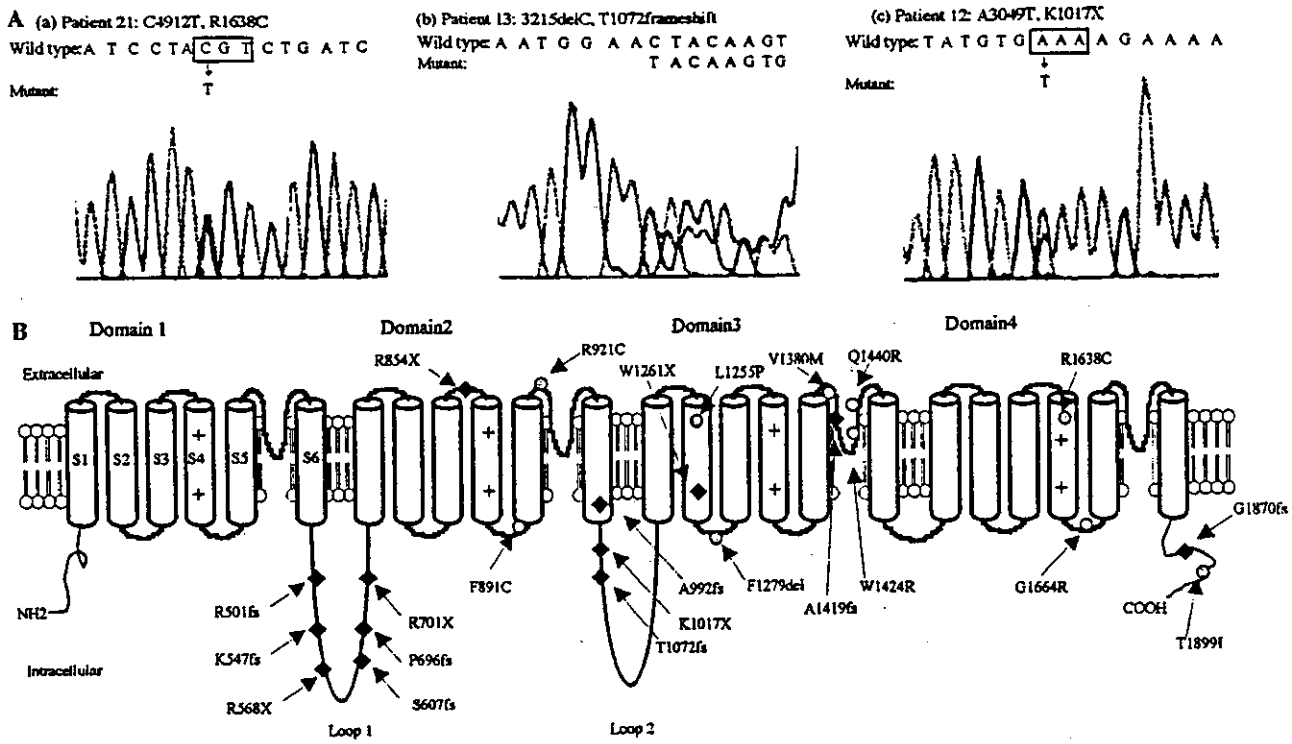

Fig. 1. Mutations in the SCN1A gene detected in the patients with SME. (A) Representative sequencing data of the SCN1A mutations. (a) Substitution of C with T at nucleotide position 4912 results in a change from arginine to cysteine at codon 1638. (b) One nucleotide deletion at nucleotide position 3215 results in frameshift at codon 1072, leading to a premature termination at codon 1077. (c) Substitution of A with T at nucleotide position 3049 leads to a change from lysine to termination codon at codon 1017. (B) Location of the mutations on the sodium channel α1-subunit. S1–S6 indicate transmembrane segments 1–6. + shows a positively charged arginine or lysine residue. Closed diamonds indicate truncation type-mutation (nonsense and frameshift mutations) and gray circles indicate missense mutation and 3-bp deletion. Each of the mutations is indicated by arrow.

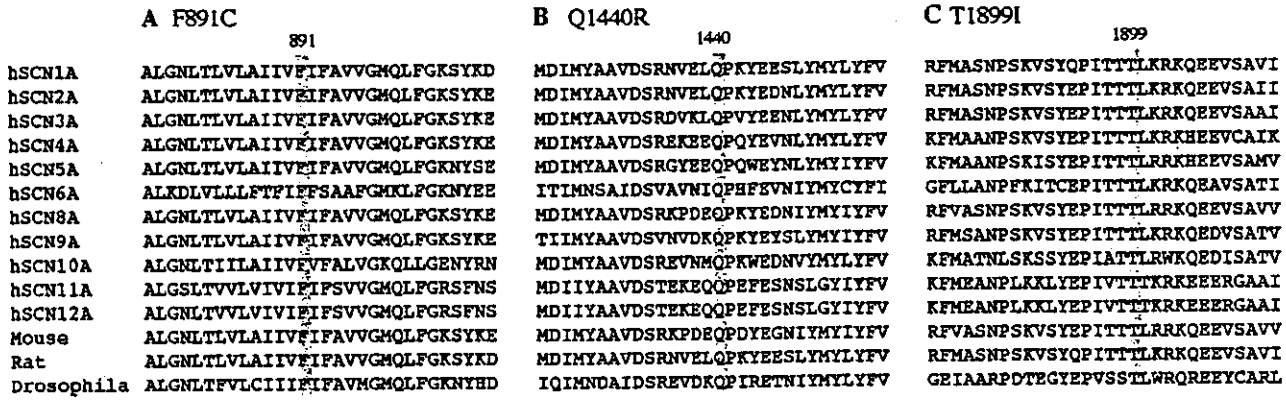

Fig. 2. Alignments of amino acid sequence in sodium channel α-subunit gene family members. Amino acids changed from phenylalanine to cysteine at codon 891 (A), from glutamine to arginine at codon 1440 (B) and from threonine to isoleucine at codon 1899 (C) are shown. GenBank Accession Nos., from top to bottom, are AF225985, M94055, AF225987, M81758, AY038064, M91556, AF225988, NM_002977, AF117907, AF188679, AF109737, 2203417A, A25019, and P35500.

As regards mutation type of the SCN1A gene in the patients with SME, 14 cases of 24 mutations were nonsense and frameshift mutations which led to some truncated proteins. It has been reported that truncation type-mutations of the SCN1A gene is related to severe phenotype in SME because all mutations in GEFS+ were missense mutations [5–10] and all but one mutations in SME were truncation type-mutation [15,16]. In our study, however, not only truncation type-mutations but also missense mutations were identified. The type of the SCN1A mutation could not be linked to the clinical severity of each patient with epilepsy.