seven participating centers. Several other patients with MCDs and putative reflex seizures were excluded because the latter were not reproduced during video-EEG evaluation. Seizure onset ranged from 4 months to 11 years (mean, 4.6 years), and age at evaluation from 5 to 51 years (mean, 20.1 years). Chart review complemented by recent clinical assessment allowed collection of data regarding pregnancy and perinatal period, developmental milestones, history of status epilepticus, reflex and spontaneous seizure types, specific precipitants of reflex seizures, occurrence of self-precipitation, seizure frequency, and response to antiepileptic drugs (AEDs). Delayed development was rated as severe when acquisition of either walking independently or comprehensive language occurred after age 3 years, and moderate when after age 2 years. Mild developmental delay included any less significant delay in acquisition of motor or cognitive milestones. Because of the ascertainment of patients from six countries, their highly variable age range, and ability to cooperate with formal neuropsychological testing, we determined the presence and severity of intellectual disability according to schooling and independence in activities of daily living. Thus patients who were able to attend normal schools but could not complete elementary education were considered mildly impaired. Those who were not able to attend regular school but were independent in activities of daily living were considered moderately impaired, and those who could not function independently were regarded as severely impaired.

Multiple sessions of 16- to 32-channel video-EEG monitoring were performed for each patient, during which reflex seizures were documented. Scalp/sphenoidal electrodes were used for these recordings, except for one patient, who was studied with subdural grids and strips in the left frontocentroparietal regions. All patients underwent complete physical and neurologic examinations. High-resolution 1.5-Tesla magnetic resonance imaging (MRI) studies were available for all. Four patients underwent surgery: two had callosotomies, and two others localized cortical resections. Routine histopathology staining was done in excised surgical specimens. Postoperative follow-up ranged from 2.5 to 12 years.

RESULTS

Illustrative case reports

Patient 1

This 15-year-old Australian adolescent was the first-born twin at 34 weeks' gestation. He was born in good condition after emergency cesarean section for breech presentation and premature labor. He had an episode of afebrile status epilepticus at age 3 years. One year later, self-induced drop attacks developed: staring at a white paper or a shiny surface would induce a tonic seizure with stiffening of the upper limbs and loss of awareness for ~10 s, occasionally associated with jerking, and followed by postictal confusion and drowsiness. These attacks increased in frequency as he grew older, with >10 per day. He succeeded persistently in evoking a seizure because of a sensation of well-being, "warm and fuzzy," as he stares. He has been instructed to sit down before provoking the episodes to prevent injuries, but unfortunately he has still sustained severe injuries because of drop attacks and prolonged generalized tonic–clonic seizures. He also has described episodes of shooting pain in the forehead with, sometimes, eye flickering for 10–15 s recurring every 5 min. Interestingly, he never had spontaneous myoclonic jerks, atonic drop attacks, or partial motor seizures.

Developmental milestones were significantly delayed. He has a moderate to severe intellectual handicap, no cutaneous or dysmorphic stigmata, but a mild generalized spasticity, with a right-sided predominance. In addition, he has cerebellar features characterized by intention tremor, dysdiadochokinesis, and ataxia. He also has tongue dyspraxia. Neurogenetic evaluation for fragile X syndrome was negative, karyotype was normal, and EEGs showed persistent focal epileptiform discharges in the right centroparietal region. MRI revealed subependymal heterotopia extending onto both temporal and occipital horns of the lateral ventricle, a right mesial occipital dysplasia, and a cerebellar hypoplasia (Fig. 1). An interictal fluorodeoxyglucose–positron emission tomography (FDG-PET) study showed a relatively decreased glucose

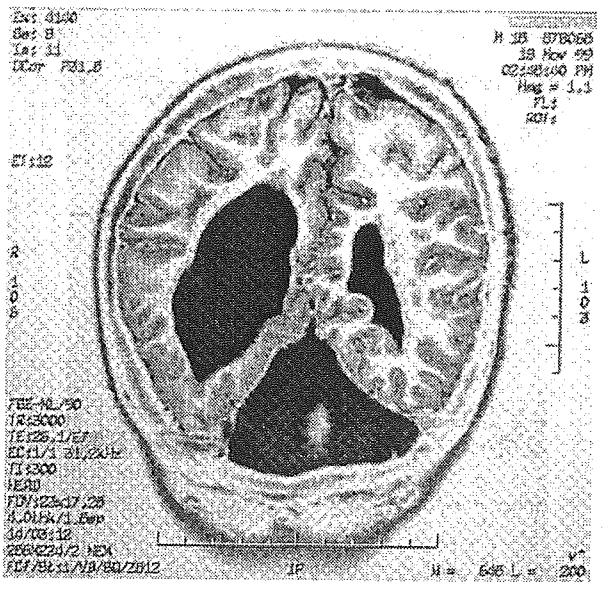


FIG. 1. Coronal inversion-recovery magnetic resonance imaging section of patient 1 shows a marked malformation in both posterior quadrants, with a right-side predominance. Note subcortical and periventricular heterotopic tissue (*arrows*), a right occipital dysplasia (*arrowhead*), dilatation of the posterior horns of the lateral ventricle, and cerebellar hypoplasia.

metabolism in right temporal, parietal, and occipital regions.

Patient 8

This 34-year-old woman had refractory seizures with onset at 8 years, which began with paresthesia and tonic extension of the right leg, and then dystonic posturing of all limbs and a high-pitched vocalization resembling a bird singing or loud laughter. Awareness was retained throughout the attacks. Seizures were spontaneous or consistently provoked by either rubbing the sole of the right foot or excessive bladder distention. Video-EEG evaluation showed interictal runs of pseudorhythmic sharp waves in the left parasagittal frontocentral region (Fig. 2A), and decremental fast activity in the same area during ictal events (Fig. 2B). MRI was normal. Intracranial evaluation with subdural strips covering the left dorsolateral superior and mesial frontocentral regions showed seizure onset in the mesial aspect of the postcentral gyrus (Fig. 2C). Extra-operative electrical cortical stimulation reproduced the seizures and confirmed that this region contained the sensory cortical representation of the right leg and foot. Resection of this region and the ipsilateral supplementary motor area, complemented by subpial transection of the mesial aspect of the motor cortex, has completely controlled seizures in the 3 years since surgery. A postoperative transient contralateral hemiparesis and expressive aphasia lasted for 1 week. A more prolonged weakness of the right foot resolved after some months with vigorous physical therapy. Postoperative MRI confirmed the extent of resection (Fig. 2D), and histopathology showed dysplastic neurons and balloon cells (Fig. 2E), indicative of a Taylor's type focal cortical dysplasia (19,20).

Clinical series of eight patients

Two patients had histories suggestive of pre- or perinatal distress, and one had status epilepticus. Five had developmental delay, and six were intellectually handicapped. Patient 2, with a deletion of the long arm of chromosome 11, had low-set ears. The other had normal physical examination. Only two patients, however, had a normal neurologic examination, with the others sharing diffuse hypotonia or spasticity, mild hemiparesis, and pseudobulbar signs. Further details can be obtained from Table 1.

Seizure characteristics

The findings are detailed in Table 2. All patients had reflex and spontaneous seizures, the latter always predominating. Seizure frequency varied from one per week to several per day. Two patients, both with severe mental retardation, had self-induced attacks.

In two patients, the semiology was identical whether attacks were spontaneous or provoked: one, with bilateral perisylvian polymicrogyria, had perioral myoclonus and drop attacks, and another, with focal cortical dysplasia in the medial frontoparietal region, had partial motor seizures and occasional secondary generalization (Tables 1 and 2). In the other six patients, the repertoire of reflex and spontaneous seizures differed in at least one seizure type. Four had spontaneous partial motor seizures and occasional secondary generalization, and recurrent drop attacks only upon specific stimulation: two with startle-induced drop attacks and two with seizures induced by visual stimulation or eye movement. A fifth patient with a centrotemporal focal dysplastic lesion had spontaneous and reflex partial motor and complex partial seizures, but perioral myoclonias only upon interoceptive stimulation of a full meal, and the last patient, with bilateral asymmetrical perisylvian polymicrogyria, had spontaneous and reflex partial motor seizures secondarily generalized, but myoclonus of the head and both arms on eating or eating-related cognitive activation.

Two patients had a single seizure-precipitating stimulus (patients 1 and 7). The others had reflex seizures induced by different types of stimuli, which nevertheless produced the same seizure manifestations. One had seizures precipitated by oculomotor and proprioceptive stimulation, two by eating or talking, two others by startle produced by sudden occurrence of a loud sound or a tactile stimulus (e.g., an unexpected stroke on the back), and one through sensory stimulation of the foot and by bladder fullness.

From clinical, interictal and ictal EEG, and imaging findings, it was possible to suggest a putative epileptogenic zone responsible for the reflex seizures in each patient. These data are detailed in Table 2. Four patients with diffuse or bilateral malformations had generalized, multifocal, or bilateral epileptiform interictal EEG spikes. All had bilateral or generalized reflex seizure patterns. Patient 2, however, with bilateral malformations in the posterior quadrants and a history of infantile spasms in the first year of life, had seizures starting in the right anterior quadrant. Three other patients had focal or unilateral spikes and localized epileptogenic zones. Patient 1 had focal EEG features associated with a gross malformation in the right posterior quadrant, although he also had bilateral periventricular nodular heterotopia. Finally, patient 7 had no interictal or unequivocal ictal epileptiform abnormalities on scalp EEGs. The autonomic features of his reflex attacks, coupled with a periinsular lesion, suggested a centroinsular epileptogenic zone.

Anatomic and histopathologic findings

MRI showed variable types of MCDs. Two patients had bilateral perisylvian polymicrogyia (Fig. 3), which was symmetrical in one and asymmetrical in the other. Both had reflex seizures provoked by eating, and one had recurrent reflex drop attacks, which were only partially responsive to anterior callosotomy. Two had extensive dysplastic features in the posterior quadrants, one with bilateral subependymal nodular heterotopia and a right occipital dysplastic cortex, and the other with grossly

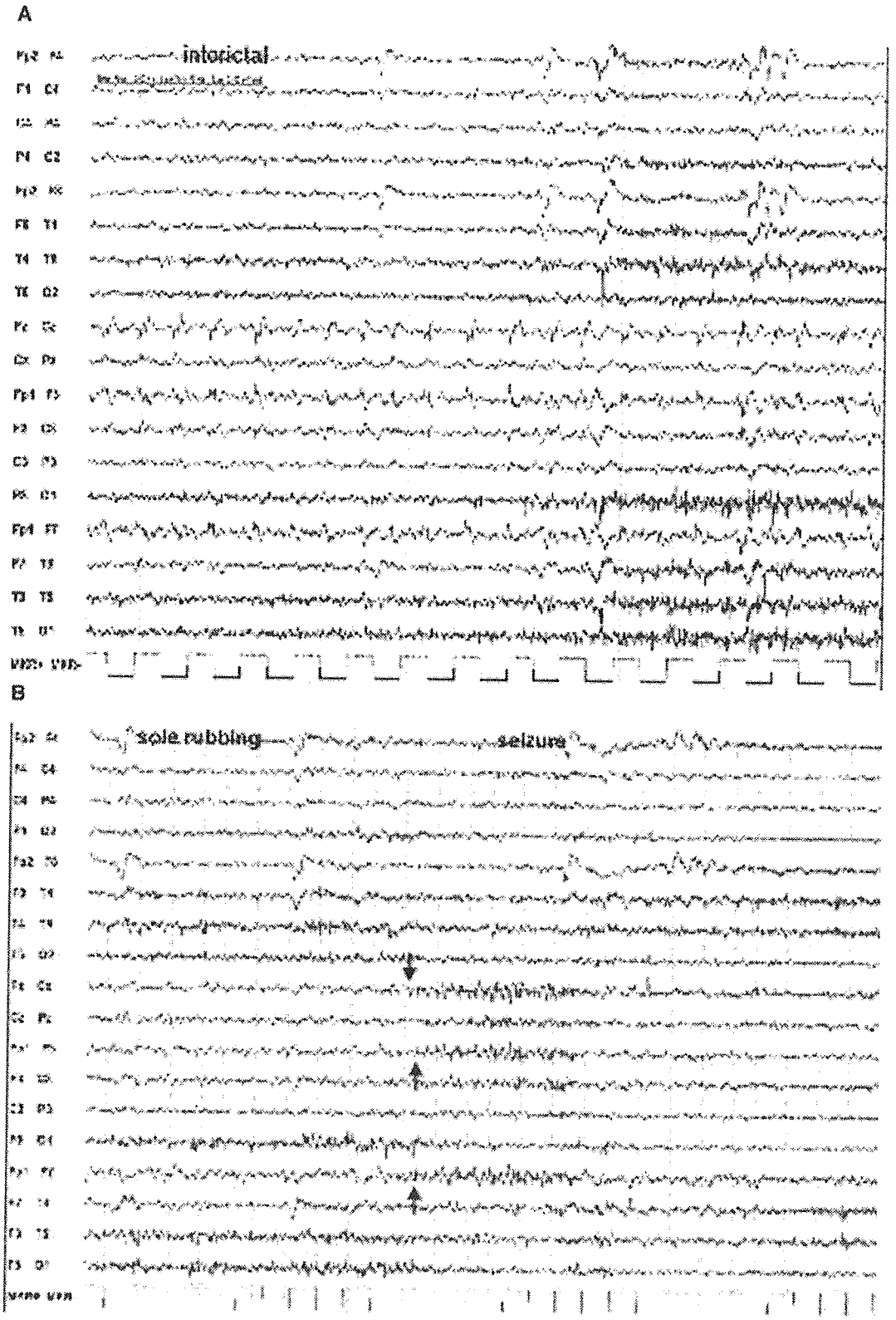
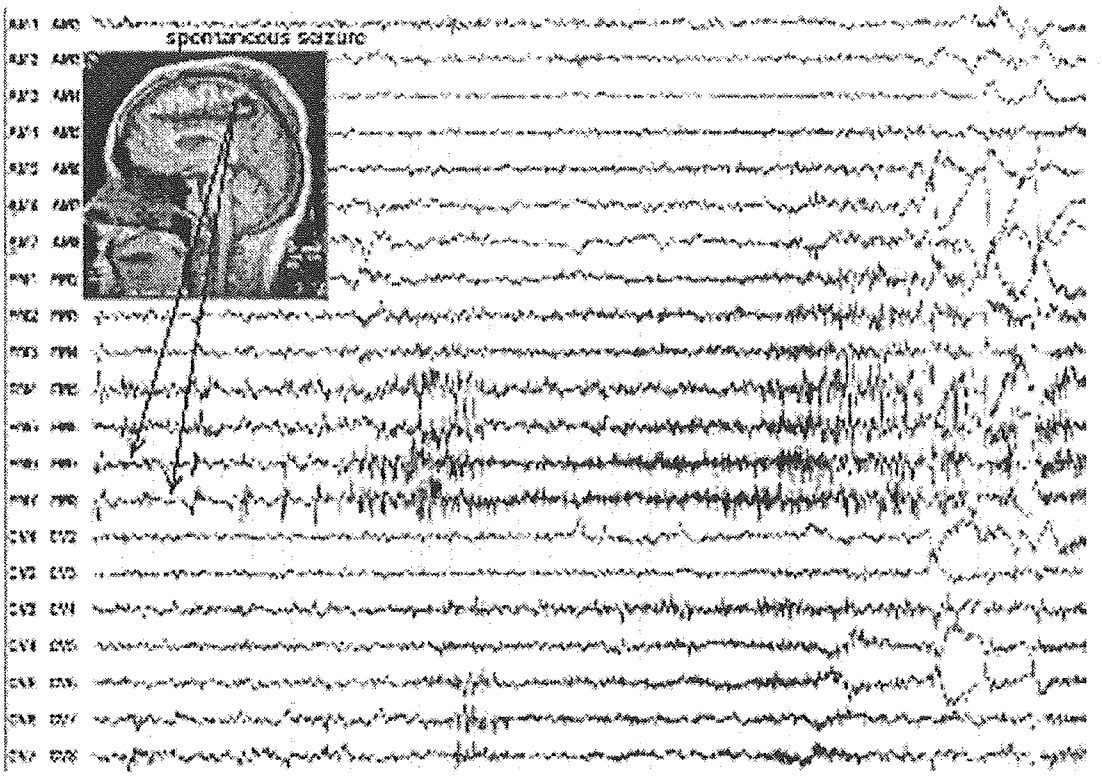


FIG. 2. EEG, magnetic resonance imaging (MRI), and histopathologic pictures of the evaluation and treatment findings of patient 8. **A:** Rhythmic interictal epileptiform discharges involving the left frontocentral (parasagittal) region. **B:** Reflex-induced seizure on scalp EEG, with onset in the same regions displaying rhythmic interictal discharges (*arrows*). **C:** Intracranial EEG with subdural electrodes (MRI insert) show a spontaneous seizure arising from the mesial primary sensory cortex, which was resected, in conjunction with the left supplementary motor area **(D)**. **E:** The dysplastic histopathologic features of the resected tissue.

C



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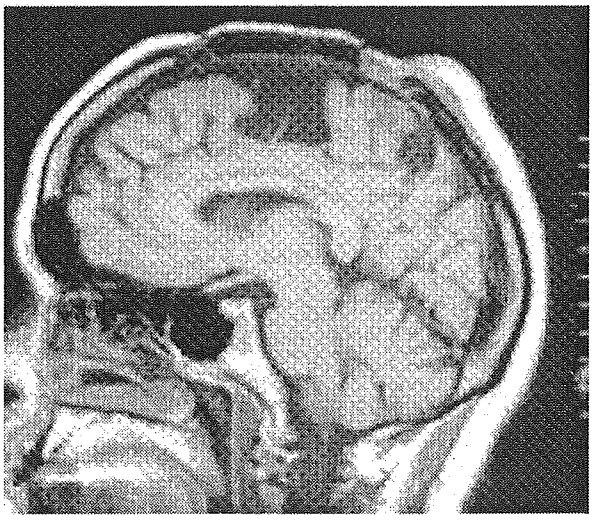

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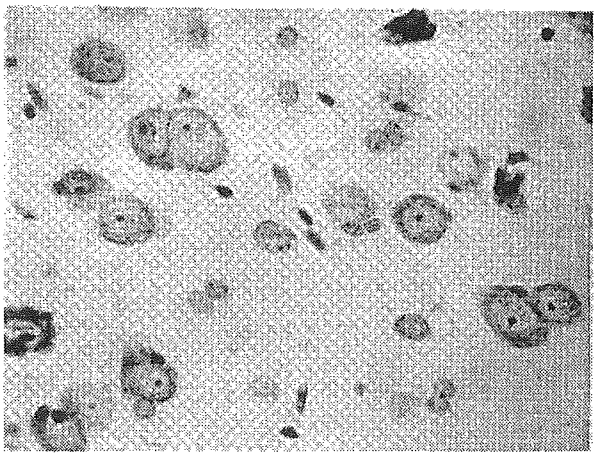

FIG. 2. Continued.

abnormal gyration in both parietooccipital regions. These patients had seizures precipitated by visual, oculomotor, or proprioceptive stimuli. A localized dysplastic abnormality was seen in two patients, in the centroinsular area in one (Fig. 4), with seizures triggered by a full stomach, and in the right frontal lobe in the other, who had startle-induced seizures. At operation, electrocorticography (ECoG) showed continuous spiking in the right frontal lobe, and histopathology confirmed a Taylor-type focal cortical dysplasia (19,20). Postoperatively, seizures were fully controlled for 5 years, and he now has occasional simple partial seizures. One male patient had diffuse band heterotopia, or "double cortex," with startle seizures and frequent drop attacks, not helped by complete callosotomy. Finally, one patient with a normal MRI, but an EEG pattern suggestive of focal cortical dysplasia (patient 8;

TABLE 1. *Clinical, anatomical, and surgical findings*

Patients	Age evaluation*	Age sz onset*	Perinatal injury	Development	Mental retardation	Abn physical exam	Abn neuro exam	MRI	Other evaluations	Surgery/ pathology	Surgical outcome
#1 Male	15	3	First twin 34 wks mild jaundice	Severely delayed	Severe	No	Gen spasticity bilat cerebellar signs	Cerebellar hypoplasia; bilat subepend heterotopia; rt occ dysp	PET: hypo-metab rt T-P-occ fragile-X excluded;	N/A	N/A
#2 Female	5	4 months	No	Severely delayed	Severe	Low implant both ears	Diffuse hypotonia	Abn gyration, both parieto-occ, rt pred	46,XX,del(11) (q22.2 q23.2)	N/A	N/A
#3 Male	7	3	No	Moderately delayed	Moderate	No	Mild lt hemiparesis	Diffuse bilat band heterotopia	N/A	Complete callosostomy	Died in status 7 years later; persisting reflex sz
#4 Male	7	3	No	Moderately delayed	Moderate	No	Diffuse hypotonia	Rt frontal blurring gray/white	N/A	Rt F lobectomy TTFCD	Free of reflex sz; mild simple partial sz persist
#5 Female	27	11	No	Mildly delayed	Mild	No	Severe pseudobulbar palsy	Bilat symm perysylvian polylicrogyria	N/A	Anterior callosotomy	Persisting reflex sz 12 years f-up
#6 Female	15	6	Bleeding first trim. gestation	Normal	No	No	Mild pseudo-bulbar palsy, oro-lingual dyspraxia	Bilateral perisylvian polymicrogyria, lt pred	N/A	N/A	N/A
#7 Male	51	3	No	Normal	Mild	No	No	Cortical thickening, increased signal inferior centro-insular	N/A	N/A	N/A
#8 Female	34	8	No	Normal	No	No	No	Normal	Subdural grid recordings left mesial central sz onset	Lt mesial F-C resection TTFCD	Sz free 30 mo f-up

*In years.

Abn, abnormal: bilat, bilateral; C, central; dysp, dysplasia; F, frontal; gen, generalized; hypometab, hypometabolism; hypsarrht, hypsarrhythmia; implant, implantation; lt, left; occ, occipital; P, parietal; PET, positron emission tomography; pred, predominant; rt, right; SBS, secondary bilateral synchrony; sz, seizure; symm, symmetrical; T, temporal.

TABLE 2. *Semilogical and neurophysiological features*

Patients	Specific precipitant	Precipitant seizure types	Interictal reflex EEG spikes	Ictal EEG	Reflex seizure putative epileptogenic zone	Seizure frequency	Self-precipitation	Spontaneous seizures	MRI
#1 Male	Staring at white paper or shiny surface	Tonic drop attacks	Rt C-P	Diffuse attenuation C-P-O pred	Rt posterior quadrant	> 10/day refractory	Yes	Eye flickering	Cerebellar hypoplasia; bilat subependymal heterotopia; rt mesial occ dysplasia
#2 Female	Forced eye dev to lt plus forced blinking	Tonic drop attacks	Gen hypsarht; later, rt F	Rt anterior quadrant	Rt anterior quadrant	Daily refractory to AED; some response to steroids	Yes	Partial motor	Abn gyration, both parieto-occ, rt pred
#3 Male	Startle touch and sound	Drop attacks	Multifocal SBS	Generalized irregular spike and attenuation	Generalized	Daily, refarctory	No	Partial motor sec gen	Diffuse bilat band heterotopia
#4 Male	Startle touch and sound	Drop attacks	Rt F-C SBS	Rt F-C	Rt F	Daily to weekly, refractory	No	Partial motor sec gen	Rt frontal blurring gray/white
#5 Female	Eating talking over the phone	Peri-oral myoclonia drop attacks	Bilat C-T SBS	Generalized rhythmic alpha-like activity	Bilat perisylvian	Daily refractory	No	Peri-oral myoclonus, drop attacks	Bilat symm perysylvian polylicrogyria
#6 Female	Eating talking about eating	Myoclonus, head and arms; partial motor sec gen	Bilat C-T lt T pred SBS	Generalized irregular spike and attenuation	Bilat perisylvian	Weekly, refractory	No	Partial motor sec gen	Bilateral perisylvian polymicrogyria, lt pred
#7 Male	Gastric dilation after meals	Peri-oral myoclonia partial motor, sec gen	None	None	Lt centro-insular	Daily to weekly, refractory	No	Partial motor complex partial, sec gen	Cortical thickening, increased signal lt centro-insular
#8 Female	Rubbing sole rt foot bladder distension	Partial motor sec gen	Continuous lt F-C	Lt medial post-central gyrus	Lt medial post-central gyrus	Daily to weekly, refractory	No	Partial motor sec gen	normal

Bilat, bilateral; dev, deviation; lt, left; occ, occipital; pred, predominant; rt, right; sec gen, secondarily generalized; symm, symmetrical.

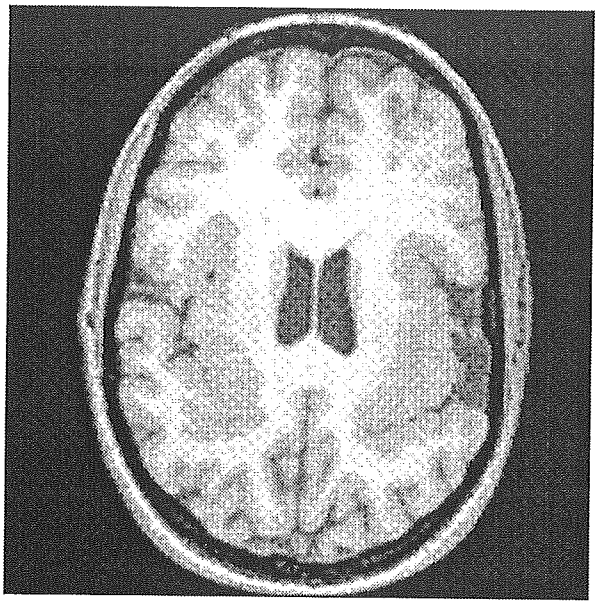

FIG. 3. Axial T_1-weighted magnetic resonance imaging section of patient 6 shows grossly malformed perisylvian regions bilaterally. Close analysis of the malformation suggests a polymicrogyric pattern.

Fig. 2A) (12,15) had seizures provoked either by stimulation of the sole of the right foot or by fullness of the bladder. Subdural electrodes showed an epileptogenic zone in the right medial central cortex, and resection of this area revealed a Taylor-type focal cortical dysplasia. Seizures are fully controlled after 30 months.

DISCUSSION

Reflex seizures result from neuronal circuits that are hyperexcitable to specific afferent stimuli (2,10,11). Genetic and lesional mechanisms facilitating the recruitment and synchronization of larger neuronal pools by incoming sensory volleys are likely to be involved in this process (11,14,16).

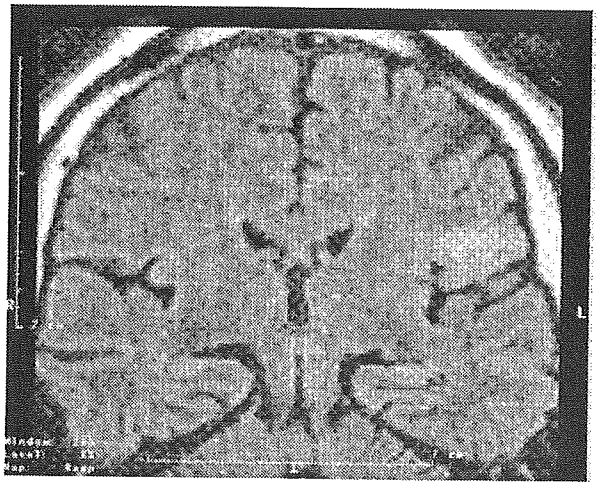

FIG. 4. Coronal fluid-attenuated inversion recovery magnetic resonance imaging acquisition of patient 7. Note focal area of increased corticosubcortical signal in the left anterior frontoinsular region, compatible with a dysplastic lesion.

We retained for inclusion in this report only those patients with MCDs who had reflex seizures reproduced during video-EEG evaluation. Other patients whose putative reflex attacks could not be verified were not included. Some of these reported attacks that were suggestive of a reflex mechanism, such as patients with rolandic MCD reporting clonic perioral movements associated with toothbrushing. Thus the very low prevalence of reflex seizures in patients with MCD, as inferred by the limited number of patients included in this report, may represent an underestimation.

All eight patients had both spontaneous and reflex seizures. This has been the case with many of the patients previously reported, irrespective of the underlying etiology (9,21). In contrast with most other reports, however, our patients had refractory seizures, and most had at least one type of seizure that was provoked only by specific stimulation and did not occur spontaneously.

Reflex seizures are often readily controlled with medication. This favorable response is to be expected in patients with idiopathic epilepsies, such as primary generalized epilepsies and reflex seizures related to photic stimuli, and also is seen in patients with partial cryptogenic epilepsies (1,3,5,6,8,22–24). Conversely, a few series and single-case reports of patients with symptomatic partial epilepsies and refractory reflex seizures have been published (9,25–28), and the present series would support the view that reflex seizures in the context of symptomatic epilepsies tend to be medically refractory.

Six of our eight patients had some types of seizures that occurred only upon specific stimulation, but not spontaneously. These were drop attacks or axial myoclonic seizures and were seen in patients with localized or more extensive MCDs. These seizure types usually result from rapid synchronization of ictal activity over both cerebral hemispheres, or from the activation of corticosubcortical circuits involved in the maintenance of axial tonus (29,30). It is possible that the enhanced epileptogenicity associated with MCDs may facilitate fast bilateral or subcortical propagation of ictal activity, leading to drop attacks or axial myoclonus upon sensory stimulation. With the exception of startle-induced seizures (26), severe seizures exclusively induced by sensory stimulation are rare, and it is thus possible that the dysplastic nature of the lesion in our patients facilitated their occurrence. [Indeed, a detailed report found that a significant number of patients with startle-induced drop attacks had variable forms of MCDs on evaluation with high-resolution imaging (26)].

A number of clinical and EEG findings suggest that MCDs are highly and intrinsically epileptogenic. These lesions often lead to medically refractory seizures (12,13), give rise to epilepsia partialis continua and other types of

status epilepticus (31–36), and produce continuous epileptogenic discharges recorded on the scalp EEG or directly over the lesion, on short- or long-term ECoG (15,16,37,38) (see Fig. 2A). Furthermore, these lesions are often localized around the rolandic regions (12,31,39), thus having the potential to lead to hyperexcitable sensorimotor synaptic loops.

The mechanisms associated with the intrinsic and enhanced epileptogenicity in MCDs are an active field of research. Morphologic studies point to persistent "epileptogenic" plasticity and abnormal connectivity, and it has been suggested that dysplastic neurons and balloon cells may escape programmed cell death through continuous expression of neurotrophins and *trk* receptor proteins (40). This would allow these cells to augment their synaptic network through retained active neurite plasticity. Neurophysiologic evidence for increased and intrinsic epileptogenicity of dysplastic tissue was recently obtained from in vitro studies of slices of tissue resected from patients with focal cortical dysplasia. In the presence of 4-aminopyridine, a K^+ channel blocker that increases transmitter release, spontaneous and prolonged epileptiform discharges resembling EEG seizures were recorded (41). These were blocked by the application of NMDA (*N*-methyl-D-aspartate) and AMPA (D-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptor antagonists, suggesting the participation of excitatory amino acid receptors in the process. Furthermore, immunocytochemical studies of malformed tissue have shown a reduced density of inhibitory γ-aminobutyric acid (GABA)ergic interneurons and an increase in the number of abnormally oriented pyramidal cells with immunostaining for excitatory amino acid receptors (42–44).

The basic mechanisms of epileptogenicity associated with other types of MCD—notably the heterotopias and polymicrogyria—have received less attention. Notably, however, it has been demonstrated that immature neurons in heterotopic positions can make reciprocal connections with the neocortex and other heterotopic aggregates (17,18), which could lead to networks of sustained hyperexcitability. In addition, basic research on animal models of polymicrogyria (45–48) has attested to the formation of hyperexcitable intracortical networks. Further studies on the neurochemical changes underlying both polymicrogyric and heterotopic malformations may clarify the epileptogenic tendency in these lesions.

The role of the different forms of MCDs in the generation of reflex seizures in our patients is additionally supported by functional anatomic correlation with the reflex seizure types (see Tables 1 and 2). Thus, patients with perisylvian lesions had seizures induced by eating or talking; those with posterior quadrant abnormalities had reflex attacks precipitated by visual, oculomotor or proprioceptive stimuli; one patient with a periinsular lesion had seizures related to interoceptive stimuli; and one of the patients with startle-induced drop attacks had a diffuse band heterotopia (the other had a diffuse frontal lobe abnormality). Finally, one patient with seizures related to somatosensory stimulation of the sole of the right foot and to bladder fullness had a dysplastic lesion in the contralateral sensorimotor cortex.

Interestingly, in many instances, these malformations retain the usual function expressed by the region where they are located or where heterotopic neurons were bound to migrate. Although much is still to be learned in terms of MCDs and cortical function, some evidence from functional MRI suggests a gradient of functionality along the spectrum of MCDs. Thus polymicrogyric lesions are most often functional, followed by heterotopia. Focal cortical dysplasia and hemimegalencephaly, conversely, are the least functional (49,50). The potential inverse relation between functionality and degree of epileptogenicity is an attractive speculation, inasmuch as converging data show that epileptogenicity is highest in focal cortical dysplasias (51).

In patients with refractory reflex seizures associated with localized MCDs, resective surgery should be considered (27), and the favorable result achieved in patient 4 supports this approach. Likewise, if a dysplastic lesion is suspected on the basis of clinical and electrographic findings in patients with reflex seizures and normal MRI, further evaluation and surgery also should be considered, as shown by patient 8. The role of callosotomy to control reflex drop attacks in these patients is less clear.

It is intriguing that in most of our patients, different types of stimuli led to the same type of reflex seizures, suggesting that different types of stimuli can be channeled to the motor regions through the same sensory pathways. The two patients with eating seizures also had the same type of reflex seizures precipitated by talking. Seizures induced by talking can involve the same perisylvian regions responsible for eating-related attacks (9,25). Similarly, the patient with seizures independently provoked both by eye deviation to the left side and by blinking most likely has a trigger related to the cortical projection of proprioceptive afferents from the ocular muscles, which could be activated by either action. In the patient with seizures induced by rubbing the sole of the right foot and by urinary bladder distention, both stimuli would project to the medial aspect of the central region, where a focal cortical dysplasia was found at surgery. Finally, in two patients, sudden, unattended, presentation of either auditory or tactile stimuli led to startle-induced seizures (21,26,52).

The low frequency of reflex seizures reported in patients with MCDs might argue in favor of possible *protective* effects secondary to the malformation. Indirect evidence supporting this possibility is that in some patients operated on for an MCD, postoperative seizures originate in regions remote from the resection site or even in the contralateral hemisphere (53–55). It is possible that parts of the MCD

exerted tonic inhibitory effects on these regions, which became active only after removal of the major MCD. This hypothesis raises the issue of "distributed epileptogenesis" in MCDs (55). By this token, epileptogenicity associated with MCD would be distributed over a network interconnecting separate "foci" with variable epileptogenic thresholds. These foci could all be within the limits of the visible lesion or involve nonlesional adjacent or distant, synaptically interconnected regions. When outside the boundaries of the visible lesion, these foci could be associated with microscopic dysplastic abnormalities or simply represent low-threshold regions that developed as such through mechanisms of secondary epileptogenesis, particularly kindling (56). In such a network, ictal and interictal activity generated within slightly distinct timeframes at different "nodes" could have a net inhibitory effect over the whole network. Recent data derived from signal analysis of ictal stereo-EEG recordings in patients with partial seizures do suggest that an epileptogenic network, rather than a single focus, is operative immediately preceding seizure onset (57–59). Interestingly, however, this network conformation also can lead to scenarios in which ictal activity locally generated at a single node of a distributed network may be prevented from recruiting the whole network (and thus lead to a clinical seizure) if other nodes are displaying high-frequency, out-of-phase interictal spiking. This has been shown to occur experimentally (60) and is a theoretical model to suggest that out-of-phase hyperexcitability within a distributed network may indeed have a net inhibitory effect over the network. Such a model of distributed epileptogenesis could provide a mechanism through which a hyperexcitable response to an afferent sensory volley may not lead to reflex seizures. The fact that MCDs are highly epileptogenic and tend to generate a large number of interictal spikes may very well fit this model. As a corollary, clinical and basic science studies of MCDs may provide an opportunity for exploring both excitatory and inhibitory influences of high degrees of epileptogenicity on epileptogenic networks.

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CASE REPORT

Herpes simplex virus type 2 recurrent meningitis (Mollaret's meningitis): a consideration for the recurrent pathogenesis

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KEYWORDS
Herpes simplex virus;
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Low immune response

Abstract We report a 44-year-old Japanese woman with herpes simplex virus (HSV) type 2 recurrent meningitis (Mollaret's meningitis). The diagnosis was confirmed by nested polymerase chain reaction in her cerebrospinal fluid, but the patient's conventional HSV antibodies by complement fixation, neutralizing test or enzyme immunoassay showed low titres with low lymphoproliferative response. Several similar cases are discussed. Although the reason for the recurrent pathogenesis is uncertain, our report suggests that the low immune response including immune evasion may be involved in the pathogenesis of HSV type 2 recurrent meningitis. For this patient, long-term suppressive and patient-initiated therapies were conducted to prevent the recurrence of meningitis.

Introduction

Mollaret's meningitis is defined as a benign recurrent aseptic meningitis characterized by three to 10 episodes of fever and signs of meningeal irritation lasting between 2 and 5 days, associated with spontaneous recovery.[1-3] To date, approximately 50 cases of herpes simplex virus (HSV) type 2 recurrent meningitis have been reported.[4-11] Polymerase chain reaction (PCR) from cerebrospinal fluid (CSF) can confirm the diagnosis in most patients, aiding further management of the patients, whereas the antibody analysis is not always useful. The pathogenesis of recurrence has

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not yet been clarified; frequently recurring meningitis with or without genital herpes is probably due to particular features of HSV type 2, or low host defence including immune evasion. Previously we reported a case of HSV type 2 Mollaret's meningitis with low antibody response.[3] We herein report an additional case of HSV type 2 recurrent meningitis, in which the serum complement fixation (CF), neutralizing test (NT) or enzyme immunoassay (EIA) antibodies to HSV were all at a low level, and in which there was a low lymphoproliferative response at the third recurrence.

Case report

A 44-year-old Japanese woman had an unremarkable past and familial histories. At the end of July 2001, the patient noticed vesicles and pain in the genital region, and visited a nearby gynecologist. She was diagnosed as having genital herpes and given a vidarabin salve and a diclofenac perioral drug. On August 5, she had severe headache, a fever of 39 °C, and vomiting, and was admitted to our University Hospital. On admission, her temperature was 37.4 °C, and pulse and respiration were normal. She revealed nuchal stiffness and lower back pain without Kernig's sign, and no abnormalities were observed in her four extremities. The patient's erythrocyte sedimentation rate was 39 mm/h, a syphilic test for syphilis was negative; a chest X-ray, electrocardiogram, and brain CT and MRI were normal. CSF contained 433/mm^3 cells, 144 mg/dl protein, and 40 mg/dl glucose; blood glucose was 79 mg/dl. The CSF cytokine measurements were: tumor necrosis factor (TNF)-α 22.5 pg/ml (normal range <4.4), interleukin (IL)-6 2030 (<3.1), interferon (IFN)-γ 74.6 (<8.0), and IL-10 149 (<2.0).

Serologic testing and PCR studies: serum EIA IgM to HSV was 1.93 (positive ≥0.8), IgG 6.3 (positive ≥2.0), NT to HSV type 1 and type 2 was <1:4, respectively, and CF was <1:4. CSF EIA IgM to HSV was 0.45, IgG 1.55, and CF to HSV was <1:1. No intrathecal antibody synthesis was observed. For the conventional CF and EIA testing, HSV type 1 antigen was used. The CF and EIA showed rather high values of varicella-zoster virus of 1:16 and 21.1, respectively. Meanwhile, serum antibody titres by type-specific ELISA kit (Focus Technologies Inc) revealed HSV type 1:0.33, HSV type 2:0.30 at the 6th illness day, HSV type 1:0.33, HSV type 2:0.30 at the 21th illness day and then 0.31 and 1.93, respectively, at the 40th illness days.

The HSV-specific 198-bp DNA band was amplified by nested PCR from initial CSF, but not from the serum. The PCR product was further digested with the restriction enzyme Bam *HI*, resulting in the identification of HSV type 2. Real time HSV PCR shows 10 000 copies/ml, and serum 500 copies/ml.

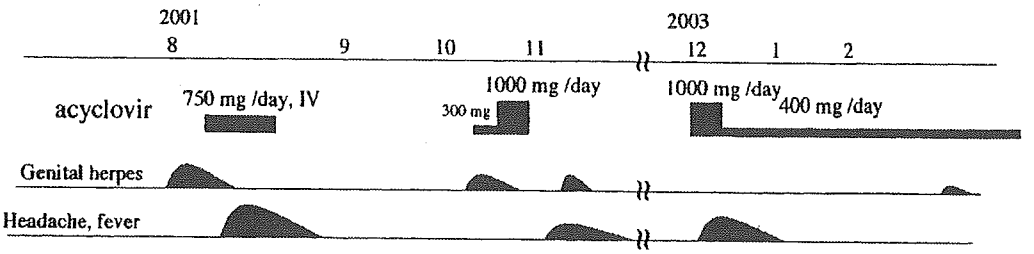
Clinical course (Fig. 1): the patient was given a 7 day-course of intravenous acyclovir 750 mg/day. The genital herpes and meningeal signs were resolved within a few days. One week later the patient's CSF pleocytosis decreased 13/mm^3, and she was discharged on August 22, however, she experienced genital herpes and headache with vomiting on September 26 and October 22. Her CSF had showed a mild pleocytosis at the latter episode. Beginning with the 2nd episode she began to have an anxiety about repeated attacks of genital herpes with meningitis, and acyclovir of 400 mg per day was periorally given as continuous suppressive therapy. In spite of this, she experienced a third attack of meningitis without skin eruption in December 2003.

On the third admission, HSV type 2 by HSV PCR and type-specific antibody analysis[12] was confirmed in the patient's CSF. Real-time PCR showed 200 copies/ml. CF, NT and EIA were low titres as observed in the first attack. Lymphocyte response to phytohemagglutinin (PHA) was 17 422 cpm (normal 26 000-53 000 cpm); concanavalin A (Con A) was 17 737 cpm (normal 20 000-48 000 cpm); CD4 was 37.7%; CD8 was 20.6%; the CD4/CD8 ratio was 1.83. After acyclovir treatment, the meningitis was resolved within a few days, and the patient returned to job, at which point she changed patient-oriented antiviral treatment; i.e. the patient initiated herself perioral acyclovir treatment as soon as she notices genital herpes or a headache indicative of meningeal sign. Several months after the third attack, PHA and Con A had normalized to 40 239 cpm and Con A: 25 216 cpm, respectively.

Discussion

In our present case of HSV type 2 Mollaret's meningitis, HSV serum antibody titers by conventional CF, NT, or EIA were at a low level during three time attacks, although type-specific HSV type 2 antibody was detected. Furthermore, a low lymphoproliferative response was found despite cytokine increases in the CSF.

As regards the recurrent pathogenesis, it has not been clarified whether HSV type 2 strain acquires a neuro-affinity in the repeated reactivation from the latent infection, or the recurrence is induced due to low host defense. Related to the former, Venot



	8/6	8/21	9/3	10/1	12/19	1/6
Serum						
HSV EIA IgM	1.93	1.80		2.02	0.45	0.40
EIA IgG	6.3	9.8		8	10.9	13.6
CF	<4	<4		<4	<4	4
HSV type 1 NT	<4			<4	<4	
HSV type 2 NT	<4			4	<4	
CSF						
Cell (/mm^3)	433	13		32	34	
Protein (mg/dl)	144	52		70	72	
HSV IgM (EIA)	0.45	0.49				
IgG (EIA)	1.55	1.53				
(CF)	<1	<1				
Real-time PCR	10,000 copies /ml				200 copies /ml	
Nested PCR	HSV-2 (+)				HSV-2 (-)	

Figure 1 Clinical course. HSV, herpes simplex virus; EIA, enzyme immunoassay; CF, complement fixation; NT, neutralizing test.

et al.[5] demonstrated that the identical HSV type 2 strain caused both meningitis and recurrent genital herpes by using PCR study with a restriction enzyme technique. In terms of the latter pathogenesis, a few of the reported cases probably showed a weak antibody response; for example, Picard et al.[6] have described one case that was merely positive for both HSV type 1 and type 2, and Cohen et al.[7] have described a case that was seronegitive. In our previous case of HSV type 2 recurrent meningitis, serum HSV antibody titers by conventional CF, CF, NT, or EIA were clearly at a low level.[3] Segawa[9] reported four cases of HSV type 2 Mollaret's, meningids with low antibody response and lymphocyte proliferation to PHA or Con A. Nakata et al. (unpublished data, 2000) described a case of idiopathic CD4 T-lymphocytopenia in HSV Mollaret's meningtis. Strong anxiety about repeated meningitis may be related to the low immune response, as shown in our present case. In addition, Segawa has mentioned a possibility of immune evasion to HSV type 2 infection.[9,13] Thus, these reports including our case strongly suggest that the low immune responses may be involved in the pathogenesis of recurrence in HSV type 2 Mollaret's meningitis.

Our present patient experienced a recurrence very soon after the initial onset. Recurrence frequently occurs within 1 year after onset. Suppressive therapy for genital herpes is becoming popular.[14,15] Prevention therapy may be necessary in the cases of frequent recurrent meningitis as well as recurrent genital herpes (Workshop 'suppressive therapy to HSV infection' Nagasaki, March 2002). For the present case, long-term suppressive and patient-oriented therapies were conducted. However, these trials were limited to a few cases. Further cases are needed to evaluate whether these therapies can prevent the recurrence of meningitis.

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LETTER

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Churg-Strauss syndrome and the leukotriene receptor antagonist pranlukast

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Abstract The authors studied eight cases of Churg-Strauss syndrome (CSS) associated with the use of pranlukast, a common cysteinyl leukotriene receptor antagonist (LTRA) in Japan. The patients who received pranlukast showed significantly increased peripheral blood eosinophil count, neurological disability scores, and poor responses to corticosteroid in comparison with those patients not receiving pranlukast. We suggest that preceding administration of pranlukast aggravates clinical presentations of CSS.

Keywords Churg-Strauss syndrome · Leukotriene receptor antagonists · Pranlukast

Abbreviations BVAS: Birmingham Vasculitis Activity Score · CSS: Churg-Strauss syndrome · LTRA: Cysteinyl leukotriene receptor antagonist · FFS: Five-factor score

Churg-Strauss syndrome (CSS) is a rare disorder characterized by asthma, peripheral hypereosinophilia, and systemic vasculitis. Its pathogenesis is not yet well established. Several recent reports have suggested an association between the use of cysteinyl leukotriene receptor antagonists (LTRAs; zafirlukast, montelukast, and pranlukast) and CSS [1–3]. Some authors hypothesized that the withdrawal or reduction in corticosteroid dose after the introduction of a LTRA leads to the development of underlying CSS [3, 4]. In contrast, cases of CSS associated with LTRAs have also been reported even for cases without corticosteroid withdrawal [5]. Since 1995, when pranlukast was introduced in Japan, there have been only five case reports of CSS associated with pranlukast [2, 6–9]. Despite these reports, the role of LTRAs in the development of CSS remains to be elucidated. To investigate the role of pranlukast in the development of CSS, we herein compared the clinical presentation and laboratory data between patients with CSS receiving pranlukast and those not receiving it.

We retrospectively analyzed 20 CSS patients diagnosed according to the American College of Rheumatology criteria [10] who were treated in our hospital between 1995 and 2003. Eight patients developed CSS after receiving pranlukast. In the same period, we also encountered 12 patients with CSS who did not receive pranlukast. The Birmingham Vasculitis Activity Score (BVAS) [11] and five-factor score (FFS) [12] were used to evaluate disease activity at the time of diagnosis. Their neurological disabilities were assessed by the modified Rankin scale [13] both at the time of diagnosis and 2 months after the initiation of treatment for CSS. Fisher's exact test was used to verify the association of the variables of interest. The nonparametric Mann-Whitney test was used to analyze continuous variables. Differences were considered to be significant when the p value was less than 0.05.

In the eight patients who received pranlukast, the onset of CSS occurred between 1 and 6 months after the initiation of pranlukast administration. Low doses of oral (prednisolone 5–10 mg/day) or inhaled corticosteroid (beclomethasone dipropionate 200–800 μg/day) were administered to five patients without withdrawal before the onset of CSS. The other three patients had not received corticosteroids prior to the development of CSS. The mean eosinophil count of these eight patients was 18.2×10^9/l (fraction, 65.4%). In contrast, patients who did not receive pranlukast had a mean eosinophil count of 8.10×10^9/l (fraction, 44.3%). Both of these two parameters significantly differed between the two groups [$p=0.04$ (count), $p=0.01$ (fraction)]. For the patients receiving pranlukast, the neurological disability scores

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Table 1 Comparison of clinical features of CSS patients who either received or did not receive pranlukast. *BA* bronchial asthma, *WBC* white blood cell, *Eo* eosinophil, *ESR* erythrocyte sedimentation rate, *MPO-ANCA* antimyeloperoxidase antibody, *PSL* prednisolone, *mPSL* methylprednisolone, *NS* not significant

Pranlukast patients	Received ($n=8$; M=6, F=2)	Did not receive ($n=12$; M=4, F=8)	p value
CSS onset (age, years)	63.0	53.5	NS
BA onset (age, years)	61.0	50.0	NS
BA duration (years)	3.0	3.0	NS
Disease activity			
BVAS	20.7	18.7	NS
FFS	0.43	0.33	NS
Laboratory findings			
WBC (/l)	27.8×10^9	18.3×10^9	NS
Eo (/l)	18.2×10^9 (65.4%)	8.10×10^9 (44.3%)	$p=0.04$ ($p=0.01$)
ESR (mm/1 h)	65.0	78.9	NS
MPO-ANCA positive	75.0%	37.5%	NS
Treatment			
Total PSL (mg)	2938	2573	NS
Total mPSL (mg)	5357	900	$p=0.04$
Clinical outcome (modified Rankin scale)			
Before treatment	4.0	2.8	$p=0.03$
After treatment	3.1	1.9	$p=0.02$

were higher than those for patients not receiving pranlukast, before and after 2 months treatment ($p=0.03$, 0.02, respectively). These CSS patients were treated mainly with oral corticosteroids (prednisolone 30–80 mg/day) with one or more courses of intravenous methylprednisolone pulse therapy (1000 mg for 3 days). Four of the patients receiving pranlukast responded poorly to corticosteroids. Hence, the addition of cyclophosphamide was necessary in three patients. The total dose of methylprednisolone was significantly higher in the patients receiving pranlukast than in those who did not receive pranlukast ($p=0.04$) (Table 1).

The patients who received pranlukast showed significantly increased peripheral blood eosinophil count, neurological disability scores, and poor responses to corticosteroid compared with those of patients not receiving pranlukast. One possibility is that the underlying disease activity of CSS is high in the patients receiving pranlukast. However, we could not find a statistically significant difference in disease activity scores (BVAS and FFS) at the time of initial diagnosis between the patients receiving pranlukast and those not receiving it. Therefore, the difference in clinical presentations and laboratory data may not reflect the underlying disease activity. These differences suggested that pranlukast directly aggravates the clinical findings of CSS.

It has been pointed out that steroid withdrawal after administration of LTRAs is one of the causal factors of CSS associated with LTRAs [3]. However, in our present patients, none of them had the dose of oral corticosteroid reduced before developing CSS. Furthermore, three of the eight patients had never received corticosteroid before developing CSS. Another possibility is that pranlukast directly precipitates CSS. LTRAs have no effect on the receptors of leukotriene B4 (LTB4), which is also a chemoattractant of eosinophils and neutrophils [14, 15]. An imbalance in activity between the receptors of LTB4 and cysteinyl leukotriene receptors may exist in patients receiving LTRAs. This imbalance in activity may precipitate CSS under similar conditions.

All of the patients developed CSS within 6 months after receiving pranlukast. Thus, we must pay particular attention to asthmatic patients who are being treated with pranlukast for at least 6 months, and also to those whose corticosteroid dose has not been reduced. We also need to further analyze additional data prospectively to elucidate whether pranlukast aggravates or initiates the development of CSS.

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An increase of oxidized coenzyme Q-10 occurs in the plasma of sporadic ALS patients

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Abstract

We have compared plasma redox status of coenzyme Q-10 in 20 sporadic amyotrophic lateral sclerosis (sALS) patients with those in 20 healthy age/sex-matched controls. A significant increase in the oxidized form of coenzyme Q-10 (sALS=109.3±95.2 nM; controls=23.3±7.5 nM, P=0.0002) and in the ratio of oxidized form of coenzyme Q-10 to total coenzyme Q-10 (%CoQ-10) (sALS=12.0±9.3%; controls=3.2±0.9%, P<0.0001) were observed. Moreover, %CoQ-10 correlated significantly with the duration of illness (ρ=0.494, P=0.0315). Our finding suggests systemic oxidative stress in the pathogenesis of sALS.

Keywords: Amyotrophic lateral sclerosis; Oxidative stress; Plasma; Redox status; Coenzyme Q-10; Antioxidant

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disorder caused by degeneration and death of motor neurons in the brainstem, spinal cord and motor cortex, leading to progressive muscle weakness and atrophy. Oxidative stress has been suggested to underlie pathogenesis of ALS inasmuch as missense point mutations occur in the copper/zinc (Cu/Zn) superoxide dismutase (SOD) gene of familial ALS patients [1,2]. Indeed, an increase in levels of protein carbonyl and nuclear 8-hydroxy-deoxyguanosine (8-OHdG) in motor cortex [3] and a decrease in glutathione peroxidase activity in precentral gyrus [4] were found in familial and sporadic ALS (sALS) patients when compared with controls. Moreover, levels of 4-hydroxynonenal [5] and 3-nitrotyrosine [6] in the cerebrospinal fluid (CSF) of sALS patients were significantly greater than those in controls. Elevated levels of thiobarbituric acid reactive substances (TBARS) [7,8] and 8-OHdG [9] were found in the plasma of sALS patients as compared with controls. Nevertheless, plasma and serum levels of antioxidants such as vitamin E [7,8,10], ascorbic acid [11], coenzyme Q-10 [7,12] and carotenoids [7,8,13] were not different between sALS patients and healthy controls. Conflicting results of antioxidant enzyme activities such as SOD and glutathione peroxidase were also obtained in the plasma or serum of ALS patients [8,14,15].

We therefore applied a sensitive and reliable method [16] for the simultaneous detection of ubiquinol-10 ($CoQH_2$-10, the reduced form of coenzyme Q-10) and ubiquinone-10 (CoQ-10, the oxidized form of coenzyme Q-10) in plasma samples from sALS patients and age/sex-matched controls. Then, we calculated the ratio of CoQ-10 to total coenzyme Q-10 (%CoQ-10) inasmuch as it is a good indicator of oxidative stress. We have shown an elevated oxidative stress in patients with hepatitis,

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Table 1
Plasma hydrophilic antioxidants, lipophilic antioxidants and total cholesterol in sALS patients and controls

	Hydrophilic antioxidants				
	Uric acid (μM)	Ascorbic acid (μM)	Unconjugated bilirubin (μM)		
Controls (n=20)	298±80	31.7±18.5	5.5±2.9		
sALS (n=20)	266±69	38.8±17.0	5.6±3.5		
	n.s.	n.s.	n.s.		
	Lipophilic antioxidants				
	Vitamin E (μM)	CoQ-10 (nM)	$CoQH_2$-10 (nM)	Total CoQ-10 (nM)	%CoQ-10 (%)
Controls (n=20)	27.4±7.3	23.3±7.5	734±243	757±247	3.2±0.9
sALS (n=20)	29.3±11.5	109.3±95.2	791±305	900±327	12.0±9.3
	n.s.	***0.0002***	n.s.	n.s.	***<0.0001***
	Lipophilic antioxidants/total cholesterol				
	CoQ-10/TC (nM/mM)	$CoQH_2$-10/TC (nM/mM)	Total CoQ-10/TC (nM/mM)	TC (mM)	
Controls (n=20)	5.2±1.1	167±52	172±53	4.5±1.1	
sALS (n=20)	26.6±24.6	185±65	211±73	4.3±0.8	
	<0.0001	n.s.	n.s.	n.s.	

Each variable is expressed as mean±S.D. Number in italic and bold show P values and significant differences compared with controls, respectively, as determined by Mann–Whitney U test. n.s.—not significant, TC—total cholesterol, %CoQ-10—CoQ-10/(CoQ-10+$CoQH_2$-10).

cirrhosis and hepatoma [17], in newborn babies [18] and in patients with Parkinson disease [19].

2. Subjects and methods

2.1. Human plasma

We recruited patients with sALS from the outpatient department of our hospital and healthy controls from community volunteers, with both groups providing written consent. The following exclusion criteria were applied to all subjects: (1) regular intake of acetylsalicylic acid, statins and antioxidants such as ascorbic acid, vitamin E and coenzyme Q-10; (2) severe obesity and malnutrition; (3) previous or present history of high alcohol intake; (4) other systemic diseases such as diabetes mellitus; and (5) hepatic diseases. Plasma samples were collected from 20 sALS patients and 20 controls. The sALS patient group consisted of 13 men and 7 women between the ages of 46 to 83 years; clinical diagnosis of sALS was based on revised El Escorial criteria [20]. The control group consisted of 13 men and 7 women between the ages of 42 to 84 years. Average ages (±S.D.) in sALS and controls were 61.2±9.5 and 62.1±12.4, respectively. There was no significant difference in age distribution between sALS patients and control volunteers. The sALS patients were evaluated with respect to the site of onset of symptoms, the duration of illness and ALS score [21]. Thirteen patients had limb onset symptoms, and seven patients had bulbar onset symptoms. Average duration of illness was 46.9±43.1 months (mean±S.D.), and average ALS score was 17.7±10.8 (mean±S.D.). The sALS patients in the present study had no abnormal findings on blood gas analyses and spirometry. They also had no findings suggesting infection on physical examination and routine blood test. Eleven of sALS patients were treated with riluzole 100 mg per day. None of ALS patients were given neurotrophic factors. Blood was drawn from fasted patients or controls between 9 and 11 a.m. using sodium heparin as an anticoagulant. Plasma was separated by centrifugation at 1500 g for 10 min and was stored at 80 °C until analysis.

2.2. Analytical procedure

Plasma levels of ascorbic acid, uric acid and unconjugated bilirubin were determined as previously described [22]. In brief, plasma (50 μl) was mixed vigorously with 200 μl of cold methanol in a 1.5-ml polypropylene tube. After centrifugation at 10,000 g for 3 min at 4 °C, 50 μl of the methanol layer (corresponding to 10 μl of plasma) was injected immediately onto high-performance liquid chromatography (HPLC) equipped with an aminopropylsilyl column (Type Supelcosil LC-NH_2, 5 μm, 250 4.6 mm i.d., Supelco Japan, Tokyo) and a UV detector (260 and 460 nm). The mobile phase consisted of methanol/40 mM sodium monobasic phosphate (9/1, v/v) delivered at 1.0 ml/min.

Plasma levels of $CoQH_2$-10, CoQ-10, vitamin E (mixture of α- and γ-tocopherols) and total cholesterol were determined by a published method [16] with modifications. In brief, plasma treated with 2-propanol were analyzed with HPLC equipped with a guard column (Type Supelguard LC-ABZ, 5 μm, 33 4.6 mm i.d., Supelco Japan), an analytical column (Type Supelcosil LC-8, 5 μm, 250 4.6 mm i.d., Supelco Japan), a reduction column (Type RC-10-1, Irica, Japan, Kyoto), a UV detector (210 nm), and an amperometric electrochemical detector (ECD; Model Σ985, Irica).

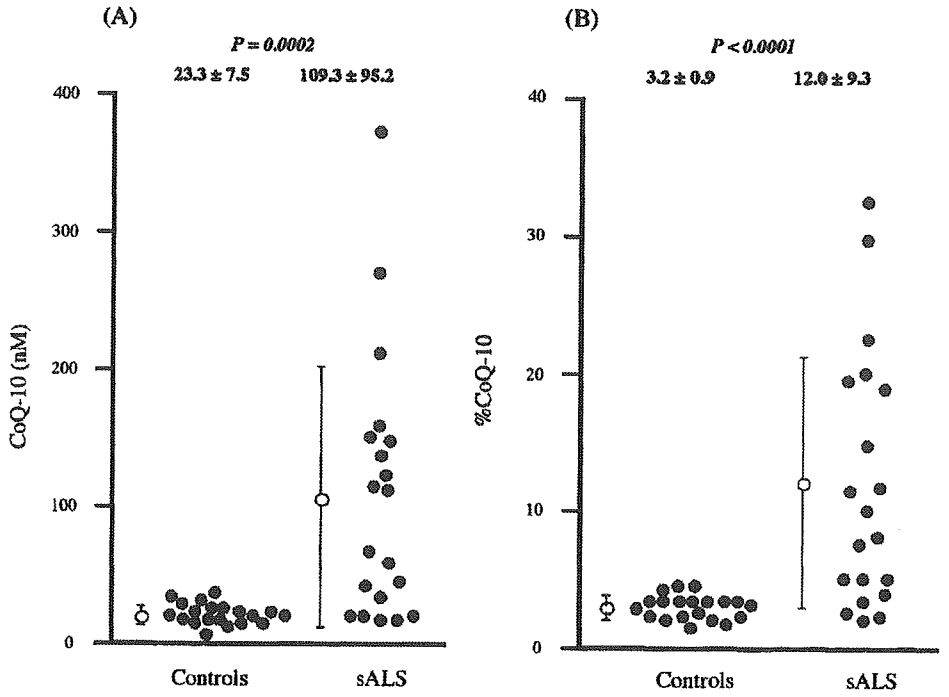

Fig. 1. (A) CoQ-10 (oxidized form of coenzyme Q-10) and (B) %CoQ-10 (the ratio of CoQ-10 to total coenzyme Q-10) in plasma from sALS patients and controls. Numbers show mean±S.D. Numbers in italic and bold show *P* values and significant differences compared with controls as determined by Mann–Whitney *U* test.

The ECD oxidation potential was +600 mV (vs. Ag/AgCl) on a glassy carbon electrode. The mobile phase consisted of 50 mM sodium perchlorate in methanol/2-propanol (9/1, v/v) delivered at 0.8 ml/min. All samples were assayed and decoded by a blinded investigator. The detection limits of uric acid, ascorbic acid, unconjugated bilirubin, vitamin E, $CoQH_2$-10, CoQ-10 and total cholesterol are 16 pmol, 2 pmol, 13 pmol, 10 fmol, 2 fmol, 2 fmol and 30 pmol, respectively. Their intraday coefficients of variation (%CV, n=4) are 1.1, 0.9, 9.1, 2.7, 2.4, 7.0 and 2.4, respectively.

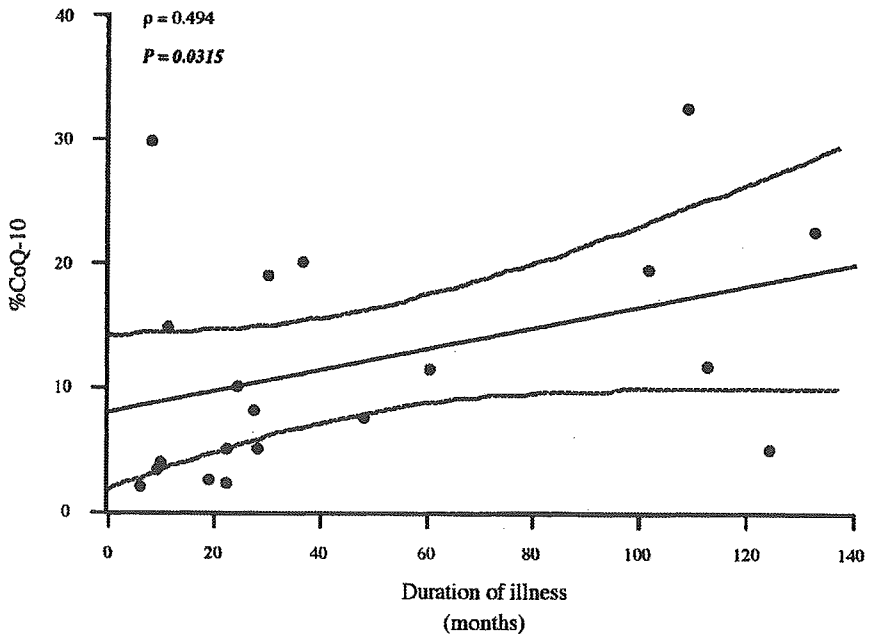

Fig. 2. Correlation between %CoQ-10 (the ratio of CoQ-10 to total coenzyme Q-10) and the duration of illness in sALS patients. Numbers in italic and bold show *P* values and significant correlation as determined by Spearman rank–order correlation coefficient. The broken lines show the 95% confidence interval.

Their interday coefficients of variation (%CV, n=4) are 2.2, 2.3, 6.0, 5.4, 3.5, 6.7 and 4.1, respectively.

2.3. Statistical analysis

The data were analyzed using StatView-J 5.0 software (SAS Institute, Cary, NC). Significant differences compared with controls were determined by Mann–Whitney U test. Spearman rank–order correlation coefficient was used to evaluate correlations between variables. The level of statistical significance was defined as P value<0.05.

3. Results

Table 1 summarizes plasma hydrophilic and lipophilic antioxidants in sALS patients and controls. The ratios of CoQ-10 and $CoQH_2$-10 to total cholesterol were determined. We found no significant differences in plasma levels of uric acid, ascorbic acid, unconjugated bilirubin, vitamin E, $CoQH_2$-10, total CoQ-10 and $CoQH_2$-10/total cholesterol between sALS patients and control volunteers. However, plasma levels of CoQ-10 (P=0.0002) and CoQ-10 /total cholesterol (P<0.0001) were significantly higher in sALS patients than those in controls. In addition, %CoQ-10 values were significantly higher (P<0.0001) in sALS patients than in controls (Fig. 1). Values of %CoQ-10 showed a fourfold increase in sALS patients as compared to controls, while no difference in %CoQ-10 value was observed between bulbar onset and limb onset (P=0.4509). A significant correlation was observed between %CoQ-10 values and the duration of illness (ρ=0.494, P=0.0315) (Fig. 2). However, there was no correlation found between %CoQ-10 values and the ALS score (ρ=0.390, P=0.0902).

4. Discussion

In the present study, we have examined the levels of several antioxidants and the redox status of coenzyme Q-10 in plasmas from sALS patients and healthy controls. No significant difference in plasma levels of hydrophilic antioxidants and vitamin E was observed between the two groups. Although the plasma level of ascorbic acid is usually a good marker of oxidative stress [22], no significant decrease in plasma level of ascorbic acid was observed in sALS patients, which is consistent with previous report [11]. Reason for this is not clear, but this may be due to the fact that the plasma level of ascorbic acid is dependent on its oral administration or that the lysis of tissue cells such as nervous and muscular cells can offset a decline in plasma level of ascorbic acid [22].

Coenzyme Q-10 has an important function as a carrier of electrons and protons in mitochondrial respiration, and reduced coenzyme Q-10 exerts strong antioxidant properties in plasma and cellular membranes. Vitamin E level did not decrease, but $CoQH_2$-10 was oxidized to CoQ-10 at an early stage in the oxidation of human plasma [23].

We are the first to examine the redox status of plasma coenzyme Q-10 in sALS patients and have observed a significant increase in plasma %CoQ-10 (Fig. 1). We applied a sensitive and reliable method for the simultaneous detection of $CoQH_2$-10 and CoQ-10 [16] in plasma samples from sALS patients and age/sex-matched controls in the present study. Our method has been already published and validated [16]. However, there has been no study that applied this method in plasma samples from sALS patients. Our data also showed a positive correlation between %CoQ-10 and duration of illness. In addition, %CoQ-10 was prone to increase with disease progression scaled by the ALS score, but the data is without statistical significance. We suggest that the plasma redox status of coenzyme Q-10 provides a measure of systemic oxidative stress in central nervous system and muscles in sALS patients.

Total CoQ-10 concentration in sALS patients was prone to be higher than that in controls, but the difference was not statistically significant (P=0.1850). However, this conjecture requires further examination. It is noteworthy that the previous studies showed no difference in total CoQ-10 concentration between the two groups [7,12].

We focused the antioxidants concentrations, especially coenzyme Q-10 and the redox status. Some investigators have already reported elevated oxidation products such as TBARS [7,8] and 8-OHdG [9] in the plasma of sALS patients as compared with controls. The redox status of the other antioxidants and the oxidation products in each sample need to be measured for the further investigation.

It is possible that medication affects plasma %CoQ-10 values. Riluzole could modify %CoQ-10 values by decreasing excitotoxicity and consequently oxidative stress in sALS patients. However, we found that patients treated with riluzole showed significantly higher %CoQ-10 values than did nontreated group (P=0.0016, Mann–Whitney U test). This may be due to the fact that riluzole group had longer duration of illness than nontreated group because a significant positive correlation was observed between %CoQ-10 and the duration of illness. However, it is difficult to recruit patients who are not undergoing riluzole therapy to examine the various severities and duration of illness as riluzole is important in the treatment of sALS patients.

Several patients in the present study were slowly progressive type of sALS and had long duration of illness. At early stage, they had the clinical features as primary lateral sclerosis or spinal progressive muscular atrophy. However, it should be noted that their clinical features were satisfied with the ALS criteria.

The present study is a small-scale comparison, and we aim to examine plasma samples from larger patient group in the future. The study focuses on the redox status of coenzyme Q-10 in human plasma due to difficulties in obtaining samples from other living tissues. In the future,