Table 1: Clinical characteristics of HHV-6 encephalopathy and febrile seizure patients.

| | HHV-6 encephalopathy | HHV-6 complex febrile seizures | Controls |
|---|---|---|---|
| Number of patients | 16 | 10 | 16 |
| Age (months) | 15.1 ± 5.4 | 12.6 ± 3.9 | 11.1 ± 10.8 |
| Sex ratio (M : F) | 8 : 8 | 5 : 5 | 11 : 5 |
| Sampling time (day of illness) | 1–8 | 1 | — |
| MRI abnormality | 14/16 | ND | ND |
| Outcome (without sequelae) | 5/16 | 10/10 | — |

HHV: human herpesvirus; No.: number; ND: not done; M: male; F: female; MRI: magnetic resonance imaging.

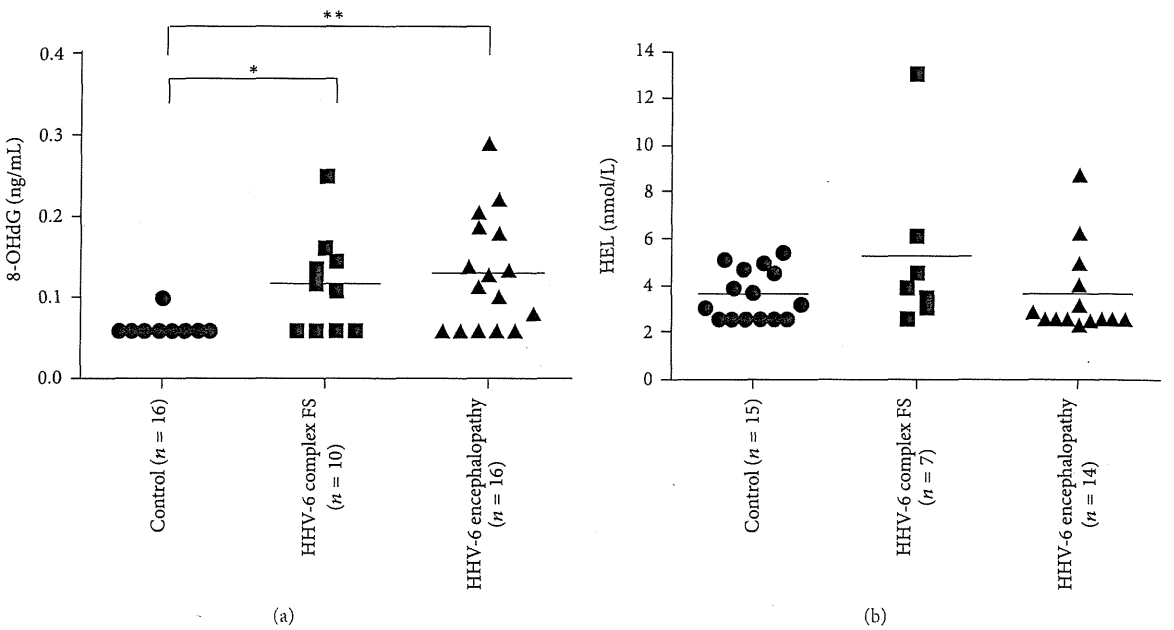


Figure 1: Cerebrospinal fluid (CSF) levels of oxidative stress markers in HHV-6 encephalopathy, HHV-6 complex febrile seizures (FS), and controls. (a) 8-hydroxy-2′-deoxyguanosine: 8-OHdG, (b) hexanoyl-lysine adduct: HEL. $^{*}P < 0.05$, $^{**}P < 0.01$. The horizontal bar indicates the mean value of each group. CSF-8OHdG levels (mean ± SD) in HHV-6 encephalopathy, HHV-6 complex FS, and controls are 0.129 ± 0.07 ng/mL, 0.116 ± 0.061 ng/mL, and 0.063 ± 0.01 ng/mL, respectively. CSF-HEL levels (mean ± SD) in HHV-6 encephalopathy, HHV-6 complex FS, and control subjects are 3.59 ± 1.87 nmol/L, 5.24 ± 3.63 nmol/L, and 3.62 ± 1.08 nmol/L, respectively.

Table 2: Descriptive statistics for the biomarkers examined[a].

| Biomarkers | Controls | HHV-6 complex FS | HHV-6 encephalopathy | Global | *P* | | |
|---|---|---|---|---|---|---|---|
| | | | | | Controls versus HHV-6 FS | Controls versus HHV-6 encephalopathy | HHV-6 FS versus HHV-6 encephalopathy |
| 8-OHdG, ng/mL | 0.063 (0.01) | 0.116 (0.061) | 0.129 (0.07) | 0.0025 | <0.05 | <0.01 | ns |
| HEL, nmol/L | 3.62 (1.08) | 5.24 (3.63) | 3.59 (1.87) | 0.1863 | ns | ns | ns |
| Tau, pg/mL | 609.0 (342.0) | 654.7 (213.7) | 13,905.6 (14,201.1) | 0.0028 | ns | <0.05 | ns |
| IL-6, pg/mL | 3.2 (3.0) | 5.8 (5.3) | 74.6 (116.9) | 0.0349 | ns | <0.01 | ns |
| IL-10, pg/mL | 0.4 (0.3) | 0.6 (0.8) | 1.4 (2.1) | 0.1663 | ns | ns | ns |
| TNF-α, pg/mL | 0.1 (0.1) | 0.3 (0.5) | 3.4 (4.0) | 0.0036 | ns | <0.01 | <0.05 |

8-OHdG: 8-hydroxy-2′-deoxyguanosine; HEL: hexanoyl-lysine adduct; HHV-6: human herpesvirus-6; FS: febrile seizure; ns: not significant; IL: interleukin; TNF: tumor necrosis factor.
[a]Values are expressed as the mean (standard deviation).

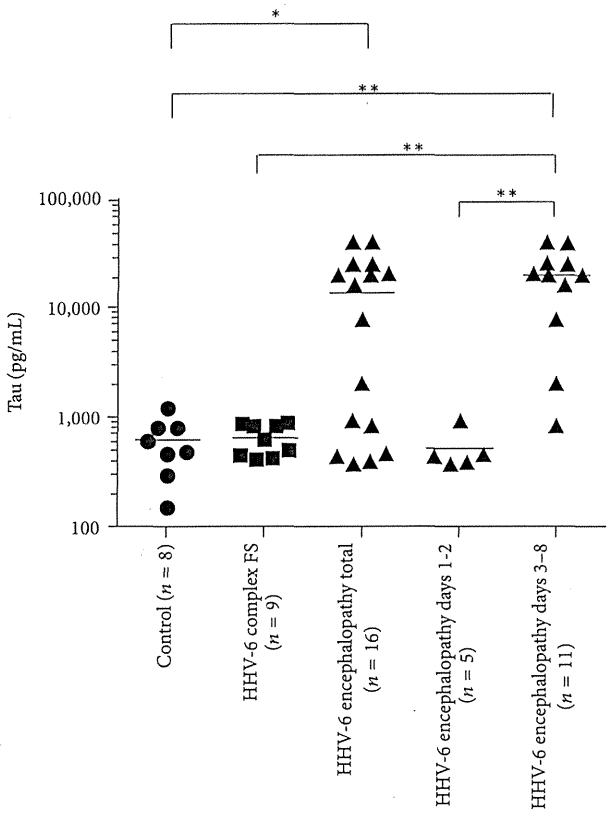


FIGURE 2: Cerebrospinal fluid (CSF) tau protein levels. The horizontal bar indicates the mean value of each group. CSF levels (mean ± SD) of tau protein in HHV-6 encephalopathy, HHV-6 complex febrile seizures (FS), and controls are 13,905.6 ± 14,201.1 pg/mL, 654.7±213.7 pg/mL, and 609.0±342.0 pg/mL, respectively. Total tau protein levels in HHV-6 encephalopathy patients are significantly higher than in control subjects ($^{*}P < 0.05$). The levels of tau protein in HHV-6 encephalopathy at days 3–8 (19,856.9 ± 13,121.9 pg/mL) are significantly higher than those of HHV-6 encephalopathy at days 1-2 (520.4 ± 229.6 pg/mL), HHV-6 complex FS, and controls ($^{**}P < 0.01$).

the HHV-6 encephalopathy group into two groups according to sampling time at days 1-2 ($n = 5$) and days 3–8 ($n = 11$), respectively. Consequently, we found that the levels of tau protein were significantly increased at days 3–8 in HHV-6 encephalopathy (19,856.9 ± 13,121.9 pg/mL, mean ± SD) compared with those of HHV-6 encephalopathy at days 1-2 (520.4 ± 229.6 pg/mL, mean ± SD) ($P < 0.01$), HHV-6 complex FS ($P < 0.01$), and controls ($P < 0.01$) (Figure 2).

*3.4. CSF Cytokine Profile in Acute Encephalopathy and Complex FS.* We next confirmed the elevation of CSF IL-6 and TNF-$\alpha$ in patients with HHV-6 encephalopathy (Figure 3). The CSF IL-6 levels in patients with HHV-6 encephalopathy (74.6 ± 116.9 pg/mL, mean ± SD) were significantly higher than in controls (3.2 ± 3.0 pg/mL, mean ± SD) ($P < 0.01$) (Figure 3(a)). The CSF TNF-$\alpha$ levels in patients with HHV-6 encephalopathy (3.4 ± 4.0 pg/mL, mean ± SD) were also significantly higher than those with complex FS (0.3 ± 0.5 pg/mL, mean ± SD) and in controls (0.10 ± 0.1 pg/mL, mean ± SD) ($P < 0.05$ and $P < 0.01$, resp.) (Figure 3(c)). In contrast, there were no significant differences of CSF IL-10 levels among patients with HHV-6 encephalopathy or HHV-6 complex FS and controls (Figure 3(b)).

*3.5. Correlation Analysis of CSF Biomarkers in HHV-6 Encephalopathy.* We next examined correlations between CSF-8OHdG and other biomarkers in the HHV-6 encephalopathy group (Table 3). There was a significant positive correlation between IL-6 and TNF-$\alpha$ (Spearman $r = 0.783$, $P = 0.0006$). However, there were no significant correlations among other biomarkers. In addition, there was no correlation between the increased levels of each biomarker and the presence or absence of neurological sequelae in HHV-6 encephalopathy (data not shown).

*3.6. Changes in CSF-8-OHdG and CSF-HEL Levels before and after Edaravone Treatment in HHV-6-Associated Acute Encephalopathy and Complex FS.* Finally, we compared the CSF levels of oxidative stress markers in six patients with HHV-6 infection (5 patients with encephalopathy and 1 patient with febrile seizures) before and after edaravone treatment. Clinical profile of patients with edaravone treatment is shown in Table 4. The mean initiation time of edaravone treatment was day 4.8 for the HHV-6 encephalopathy group. One patient with febrile seizures associated with HHV-6 infection who received edaravone treatment from day 1 did not develop encephalopathy and recovered without sequelae (patient 6). The CSF-8-OHdG levels decreased after edaravone treatment ($P = 0.0202$, paired $t$-test) (Figure 4(a)). Regarding the CSF-HEL levels, there were no significant differences between before and after edaravone treatment. We also compared the mean CSF levels of other biomarkers before and after treatment and observed no significant differences of mean values (data not shown).

## 4. Discussion

In the present study, we demonstrated that CSF-8-OHdG levels in HHV-6 encephalopathy and HHV-6 complex FS patients were significantly higher than in controls, suggesting increased oxidative stress is induced by HHV-6 infection. Recent studies revealed that oxidative damage is an emerging general mechanism of nervous system injury caused by viral infection. For example, oxidative injury is a component of acute encephalitis caused by herpes simplex virus type 1 (HSV-1) [16]. HSV-1 infection of nervous system tissues in mice was associated with the expression of inducible nitric oxide synthase (iNOS) and the release of cytokines including TNF-$\alpha$ from inflammatory cells. Thus, increased generation of ROS and RNS can be caused by the direct effects of virus on cells and the indirect effects of host inflammatory responses [17]. Regarding HHV-6 infection, Fukuda et al. reported that urinary 8-OHdG concentrations in a patient with HHV-6 encephalopathy on the first day of hospitalization were 1.5 times higher than the mean concentration

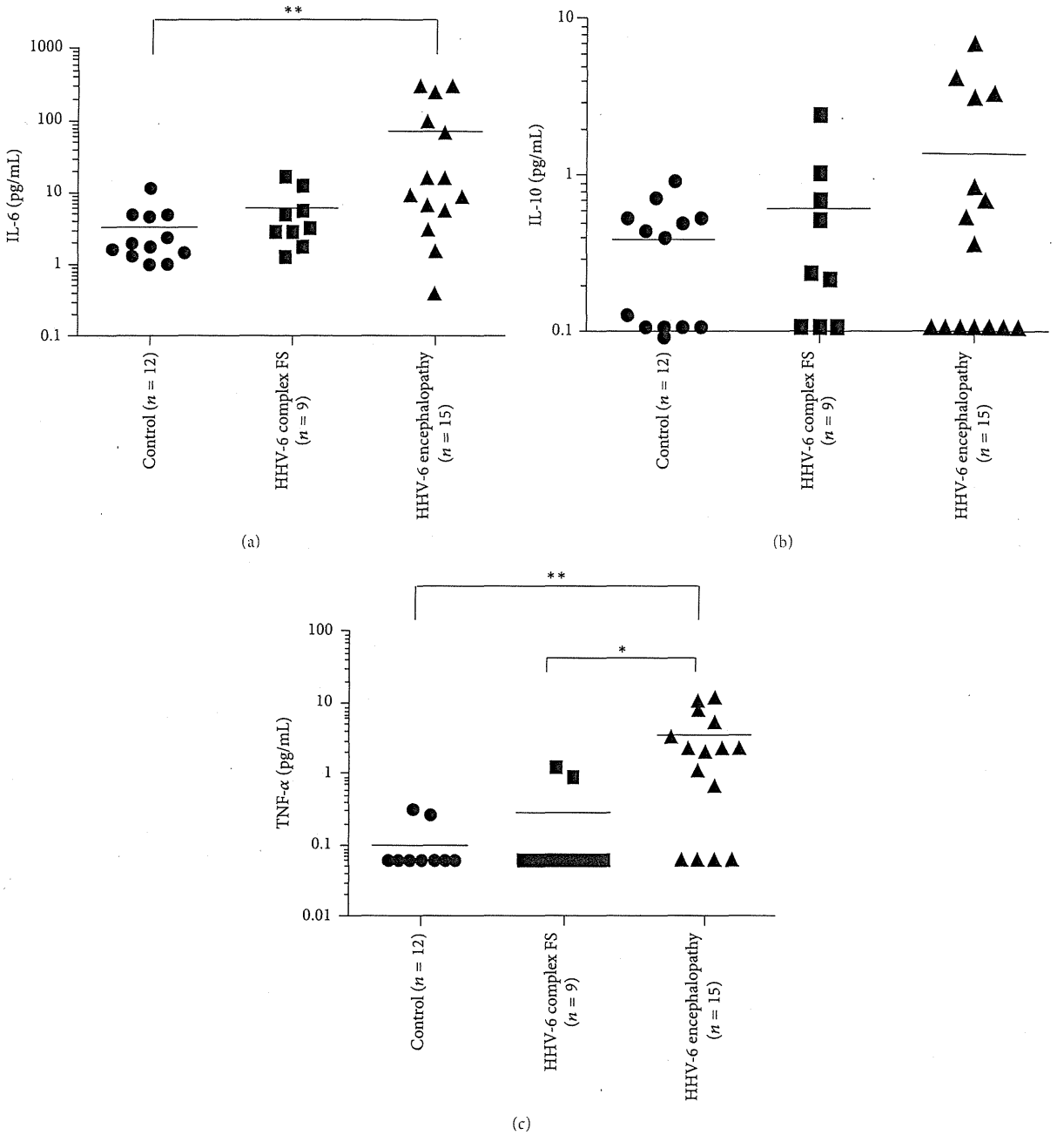


FIGURE 3: Cerebrospinal fluid (CSF) cytokine levels. The horizontal bar indicates the mean value of each group. (a) Levels of CSF IL-6 in patients with HHV-6 encephalopathy, HHV-6 febrile seizures (FS), and controls are 74.6 ± 116.9 pg/mL, 5.8 ± 5.3 pg/mL, and 3.2 ± 3.0 pg/mL, respectively. The CSF IL-6 levels in patients with HHV-6 encephalopathy are significantly higher than in controls ($^{**}P < 0.01$). (b) Levels of CSF IL-10 in patients with HHV-6 encephalopathy, HHV-6 febrile seizures (FS), and controls are 1.4 ± 2.1 pg/mL, 0.6 ± 0.8 pg/mL, and 0.4 ± 0.3 pg/mL, respectively. (c) Levels of CSF TNF-α in patients with HHV-6 encephalopathy, HHV-6 febrile seizures (FS), and controls are 3.4 ± 4.0 pg/mL, 0.3 ± 0.5 pg/mL, and 0.1 ± 0.1 pg/mL, respectively. The CSF TNF-α levels in patients with HHV-6 encephalopathy are significantly higher than those with complex FS and in controls ($^{*}P < 0.05$ and $^{**}P < 0.01$, resp.).

Table 3: Correlation analysis of CSF biomarkers in HHV-6 encephalopathy.

| Biomarker | | 8-OHdG | HEL | Tau | IL-6 | IL-10 | TNF-$\alpha$ |
|---|---|---|---|---|---|---|---|
| 8-OHdG | Spearman $r$ | 1.000 | −0.292 | −0.239 | −0.484 | −0.046 | −0.315 |
| | $P$ | <0.0001 | 0.312 | 0.373 | 0.068 | 0.871 | 0.253 |
| HEL | Spearman $r$ | −0.292 | 1.000 | 0.277 | 0.476 | −0.286 | 0.497 |
| | $P$ | 0.312 | <0.0001 | 0.338 | 0.086 | 0.322 | 0.070 |
| Tau | Spearman $r$ | −0.239 | 0.277 | 1.000 | −0.036 | −0.224 | 0.091 |
| | $P$ | 0.373 | 0.338 | <0.0001 | 0.899 | 0.422 | 0.748 |
| IL-6 | Spearman $r$ | −0.484 | 0.476 | −0.036 | 1.000 | 0.226 | 0.783 |
| | $P$ | 0.068 | 0.086 | 0.899 | <0.0001 | 0.418 | 0.0006 |
| Il-10 | Spearman $r$ | −0.046 | −0.286 | −0.224 | 0.226 | 1.000 | 0.166 |
| | $P$ | 0.871 | 0.322 | 0.422 | 0.418 | <0.0001 | 0.555 |
| TNF-$\alpha$ | Spearman $r$ | −0.315 | 0.497 | 0.091 | 0.783 | 0.166 | 1.000 |
| | $P$ | 0.253 | 0.070 | 0.748 | 0.0006 | 0.555 | <0.0001 |

CSF: cerebrospinal fluid; HHV: human herpesvirus; 8-OHdG: 8-hydroxy-2′-deoxyguanosine; HEL: hexanoyl-lysine adduct; IL: interleukin; TNF: tumor necrosis factor.

Table 4: Clinical profile of patients receiving edaravone treatment.

| Patient | Clinical diagnosis | Age/sex | Initiation and dosage of edaravone treatment | Other treatments | Outcomes |
|---|---|---|---|---|---|
| 1 | HHV-6 encephalopathy | 14 m/M | Day 5<br>0.5 mg/kg × 2/day × 7 days | Mannitol, Dexamethasone, Ganciclovir, MDL, PHT | Intellectual disability |
| 2 | HHV-6 encephalopathy (AESD) | 12 m/F | Day 5<br>0.5 mg/kg × 2/day × 8 days | Mannitol, Dexamethasone, Ganciclovir, DZP, MDL | Without sequelae |
| 3 | HHV-6 encephalopathy | 14 m/M | Day 4<br>0.5 mg/kg × 2/day × 10 days | Mannitol, Dexamethasone, Ganciclovir/acyclovir, DZP, MDL | Hemophagocytic syndrome<br>Died of fulminant hepatitis |
| 4 | HHV-6 encephalopathy (AESD) | 20 m/F | Day 7<br>0.5 mg/kg × 2/day × 7 days | Mannitol, Dexamethasone, Aciclovir, MDL | Lt hemiparesis |
| 5 | HHV-6 encephalopathy (AESD) | 12 m/M | Day 3<br>15 mg/day × 10 days | DZP, MDL, steroid pulse therapy, mild therapeutic hypothermia | Moderate psychomotor retardation |
| 6 | HHV-6 febrile seizures | 10 m/F | Day 1<br>0.5 mg/kg × 2/day × 12 days | Mannitol, Dexamethasone, Ganciclovir/acyclovir, DZP, MDL, PHT | Without sequelae |

m: months; M: male; F: female; DZP: diazepam; MDL: midazolam; PHT: phenytoin; Lt: left; HHV: human herpesvirus; AESD: acute encephalopathy with biphasic seizures and late reduced diffusion.

in healthy children and they peaked at the second seizures [18]. They speculated that 8-OHdG was produced by ROS from cytokines associated with inflammation and apoptosis following brain edema because changes in urinary 8-OHdG levels reflected the degree of brain edema. However, we found that increased levels of 8-OHdG were observed not only in HHV-6 encephalopathy but also in complex FS associated with HHV-6 infection. We also showed that CSF IL-6 and TNF-$\alpha$ levels were elevated only in the HHV-6 encephalopathy group, but not in the HHV-6 complex FS group. In addition, we analyzed correlations among biomarkers and observed no significant correlations between increased 8-OHdG levels and cytokine production or increased tau levels. These results suggest that oxidative DNA damage in the brain caused by HHV-6 infection may be independent of inflammatory reactions and subsequent axonal damage.

In contrast with the increased levels of 8-OHdG, there was no significant increase of CSF-HEL levels in HHV-6 encephalopathy compared with HHV-6 complex FS and controls. We previously demonstrated that oxidative stress

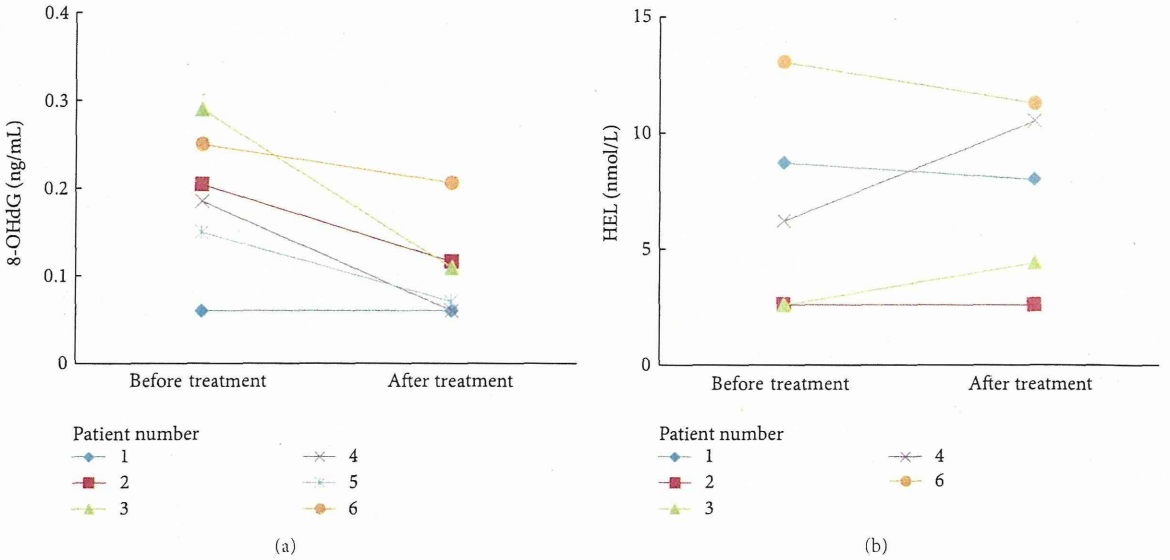


FIGURE 4: Changes in cerebrospinal fluid (CSF)-8-OHdG (a) and CSF-HEL (b) levels before and after edaravone treatment in HHV-6-associated acute encephalopathy and complex febrile seizure (FS) patients. CSF-8-OHdG levels are decreased after edaravone treatment ($P = 0.0202$, paired $t$-test).

of DNA contributes to early neuronal damage, whereas lipid peroxidation is related to subsequent neurodegeneration in subacute sclerosing panencephalitis [8]. In the present study, we only examined levels of CSF biomarkers in the acute phase of the diseases. Further investigation is required to clarify whether lipid peroxidation may be involved in the chronic phase.

Most patients with HHV-6 encephalopathy in this study (81.3%) were AESD, which has a high incidence of neurological sequelae. We previously indicated that levels of CSF tau protein were elevated in patients with AESD [19]. The current study demonstrates that CSF levels of tau protein were significantly increased at days 3–8 in HHV-6 encephalopathy compared with those of HHV-6 encephalopathy at days 1-2, HHV-6 complex FS, and controls. As CSF tau protein is considered a useful biomarker of axonal damage [20], our results raise the possibility that the high incidence of neurological sequelae in AESD is attributable to axonal injury. However, there was no correlation between the increased levels of tau protein and the presence or absence of neurological sequelae. These findings suggest that tau protein is a sensitive biomarker that might help diagnose HHV-6 encephalopathy, but it is difficult to make an early diagnosis for acute encephalopathy using this biomarker. In terms of the prognostic prediction for HHV-6 encephalopathy, another biomarker will be required because increased levels of tau protein do not always reflect a poor prognosis.

Edaravone is a free radical scavenger that interacts biochemically with a wide range of free radicals [21]. In experimental models, edaravone protects against apoptotic neuronal cell death and improves cerebral function after traumatic brain injury (TBI) [21]. In addition, Ohta et al. reported that administration of edaravone to mice immediately after TBI suppressed traumatic axonal injury and oxidative stress, which protected against trauma-induced memory deficits [22]. Edaravone is used clinically in Japan for the treatment of acute ischemic stroke. Although childhood ischemic stroke is different than in adults, the use of edaravone was recently approved for the treatment of stroke in children. In the present study, we reported 5 cases of edaravone treatment for HHV-6-associated acute encephalopathy and one case of HHV-6 complex FS. Our study is very preliminary and it is likely that the efficacy of edaravone treatment in combination with other therapies at this time was poor.

There were several limitations in this study. First, the initiation of edaravone treatment was delayed in HHV-6 encephalopathy because it is difficult to distinguish HHV-6 encephalopathy from HHV-6 complex FS during the initial seizures. Early diagnosis of HHV-6 encephalopathy, especially AESD, will be required to overcome this problem. Second, a clinical trial of edaravone for the treatment of acute encephalopathy might be difficult ethically, as placebo control cannot be used because of the severe nature of this disease. We confirmed that CSF-8-OHdG levels decreased after edaravone treatment, although there were no significant differences of mean values of other biomarkers between before and after the treatment. These results suggest edaravone treatment was partially effective for HHV-6 encephalopathy. Although these findings are encouraging, the therapeutic implications of ROS and RNS scavengers are complex, owing to their potential to exert toxic as well as protective effects [23].

## 5. Conclusion

In summary, we found oxidative DNA damage is involved in acute encephalopathy/febrile seizures associated with HHV-6 infection and may be independent of inflammatory reactions and subsequent axonal damage.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

## Acknowledgments

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Original article

# Microarray analysis of 50 patients reveals the critical chromosomal regions responsible for 1p36 deletion syndrome-related complications

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**Abstract**

*Objective:* Monosomy 1p36 syndrome is the most commonly observed subtelomeric deletion syndrome. Patients with this syndrome typically have common clinical features, such as intellectual disability, epilepsy, and characteristic craniofacial features.

*Method:* In cooperation with academic societies, we analyzed the genomic copy number aberrations using chromosomal microarray testing. Finally, the genotype–phenotype correlation among them was examined.

*Results:* We obtained clinical information of 86 patients who had been diagnosed with chromosomal deletions in the 1p36 region. Among them, blood samples were obtained from 50 patients (15 males and 35 females). The precise deletion regions were successfully genotyped. There were variable deletion patterns: pure terminal deletions in 38 patients (76%), including three cases of mosaicism; unbalanced translocations in seven (14%); and interstitial deletions in five (10%). Craniofacial/skeletal features, neurodevelopmental impairments, and cardiac anomalies were commonly observed in patients, with correlation to deletion sizes.

*Conclusion:* The genotype–phenotype correlation analysis narrowed the region responsible for distinctive craniofacial features and intellectual disability into 1.8–2.1 and 1.8–2.2 Mb region, respectively. Patients with deletions larger than 6.2 Mb showed no ambulation, indicating that severe neurodevelopmental prognosis may be modified by haploinsufficiencies of *KCNAB2* and *CHD5*, located at 6.2 Mb away from the telomere. Although the genotype–phenotype correlation for the cardiac abnormalities is unclear, *PRDM16*, *PRKCZ*, and *RERE* may be related to this complication. Our study also revealed that female patients who acquired ambulatory ability were likely to be at risk for obesity.


*Keywords:* 1p36 deletion syndrome; Chromosomal deletion; Genotype–phenotype correlation; Intellectual disability; Ambulation; Epilepsy; Distinctive features

## 1. Introduction

Monosomy 1p36 syndrome is a congenital malformation syndrome caused by the subtelomeric deletion of the short arm of chromosome 1 [1–3]. This syndrome is the most commonly observed subtelomere deletion syndrome, with an estimated incidence of 1:5000–1:10,000 [4,5]. Patients with this syndrome exhibit common clinical features, including intellectual disability (ID) and characteristic craniofacial features; such as straight eyebrows, deep-set eyes, epicanthus, and a pointed chin [6–9]. Although the levels of ID vary among patients, craniofacial features are commonly seen [10]. The patients with the 1p36 deletion syndrome also show many other complications, including hypotonia, seizures, hearing loss, structural heart defects, cardiomyopathy, ophthalmological abnormalities, and behavior abnormalities [7]. Recent advances in microarray-based chromosomal testing have helped us to identify small chromosomal rearrangements that are invisible by conventional G-banded chromosomal tests/karyotyping [11,12]. Using this method, the precise locations of the aberrations can be revealed at the molecular level. These advances have also allowed the study of more in-depth genotype–phenotype correlations for this syndrome, as well as the identification of some of the regions responsible for individual complications [12,13].

In this study, we performed a nation-wide survey for the 1p36 deletion syndrome in Japan. The aim of this study was to identify the chromosomal regions responsible for individual complications in patients with 1p36 deletions. We analyzed the affected genomic regions in 50 patients with 1p36 deletions, and performed correlational analyses of the genotype data with the clinical information.

## 2. Materials and methods

### 2.1. Patients and samples

We performed a nation-wide survey for the 1p36 deletion syndrome with the cooperation of two academic societies; the Japanese Society of Child Neurology and the Japan Society of Pediatric Genetics. The study subjects were Japanese patients who had already been diagnosed using various diagnostic methods, including conventional karyotyping, subtelomere fluorescence *in situ* hybridization (FISH) analysis, multiplex ligation-dependent probe amplification (MLPA), and chromosomal microarray testing. Five patients

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(patient [Pt] 8, 9, 21, 28, and 43), whose clinical features have been previously reported [14–17], were also included in this study. With the questionnaire survey for attending physicians, we accumulated the patients' clinical information, including craniofacial/skeletal features, neurodevelopmental features, brain structural abnormalities, cardiac abnormalities, sensory-organs abnormalities, urogenital abnormalities, endocrinological and nutritional findings among others. This study was approved by the ethics committee in Tokyo Women's Medical University.

On receipt of written informed consents from the families of the patients, we obtained the patients' blood samples to determine genomic copy number losses in the patients. Genomic DNA was extracted from the blood samples using the QIA quick DNA Extraction Kit (QIAGEN, Hamburg, Germany). Metaphase spreads were also prepared from blood samples and used for FISH analyses. In cases, if we could obtain written informed consent, parental samples were also analyzed.

### 2.2. Molecular and cytogenetic analyses

Chromosomal microarray testing was performed using any of the Agilent Oligo Microarray Kits 44, 60, 105, 180, and 244 K (Agilent Technologies, Santa Clara, CA), as described previously [18,19]. Genomic copy number aberrations were visualized using Agilent Genomic Workbench version 6.5 (Agilent Technologies). For cases in which variations of unknown significance were identified or suspected, parental samples were also analyzed. In cases of complex chromosomal rearrangements or mosaicism, metaphase spreads prepared using the patients' samples were used for FISH analyses for confirmation. The bacterial artificial clones were selected from the UCSC genome browser (http://genome.ucsc.edu/) for use as probes. For the target probes, RP11-425E15 (1p36.33: 949,400–1,132,489), RP11-82D16 (1p36.33: 2,046,751–2,208,312), RP11-70N12 (1p36.32: 2,740,703–2,922,551), CTD-3209F18 (1p36.32: 3,530,092–3,769,006), and RP11-933B18 (1p36.31: 5,988,719–6,177,261) were selected, while CTB-167K11 (1q44: 249,250,621–249,250,621) was used as a marker of chromosome 1. All of the genomic regions are described according to the February 2009 human reference sequence (GRCh37/hg19) in this study.

## 3. Results

### 3.1. Molecular-cytogenetic findings

We obtained clinical information from 86 patients with chromosomal deletions involving 1p36 regions. Among them, 50 patients (15 males and 35 females) were successfully genotyped. All of the genotypes were summarized in Tables 1 and 2, and 1p36 deletions identified in the patients were depicted in the genome map (Fig. 2). The minimum and maximum deletion sizes was 0.9 and 12.9 Mb, respectively. Pure terminal deletions were identified in 38 patients (76%). Among them, three patients (Pt 8, 19, and 21) exhibited mosaicism. Pt 8 was first diagnosed with mosaic 1p36 deletion by chromosomal microarray testing, and Pt 21 had been diagnosed with 1p36 deletion using subtelomere FISH analysis; however, mosaicism was not reported at that time [17]. Although the mosaic deletion of 1p36 in Pt 19 had been firstly confirmed by FISH, we could not detect the breakpoint by chromosomal microarray testing due to low frequency (28% mosaic ratio). As the breakpoint was determined to be between two FISH probes (CTD-3209F18 and RP11-933B18), the proximal end of CTD-3209F18 was used as the minimum deletion region in this patient.

Additional aberrations with the sizes over 0.5 Mb were identified in eight patients (Pt 2, 10, 11, 15, 20, 28, 34, and 43) involving chromosomes 4, 7, 8, 13, and Y (Table 2), including a possible benign copy number aberration in Pt 15, which was also observed in the healthy mother. The other seven patients were confirmed to have unbalanced translocations by cytogenetic evaluation (14%), using either G-banding or FISH analysis. Two translocations were diagnosed as de novo, and the others were designated as unknown because of the lack of availability of parental information.

Five patients (Pt 1, 14, 47, 48, and 50) had interstitial deletions (10%) with a deletion size between 0.9 and 10.3 Mb.

### 3.2. Clinical findings

Clinical information of the 50 patients successfully genotyped is summarized in Table 3. Estimated frequencies of each complication are also included in Table 3. Pt 26 and 49 suddenly died at 24 and 10 months old of age, respectively. Pt 49 probably died due to heart failure but Pt 26 died of an unknown cause (detailed information unavailable).

#### 3.2.1. Craniofacial features

Most of the patients showed craniofacial features, including straight eyebrows (84%), deep-set eyes (93%), broad nasal bridge (97%), low set ears (88%), and a pointed chin (89%). Constellations of these findings make distinctive facial impressions for 1p36 deletion syndrome, observed in Pt 3, 6, and 14 (Fig. 1b–d). This observation suggests that hypotelorism is rather characteristic among these patients. On the other hand, Pt 1 did not show deep-set eyes (Fig. 1a). The craniofacial features of three patients (Pt 47, 48, and 50) did not exhibit hypotelorism (Fig. 2e–g). From the genotypic point of view, these three patients (Pt 47, 48, and 50) would be diagnosed as having the proximal 1p36 deletion syndrome [20,21].

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Table 1
The ranges of 1p36 deletions analyzed by chromosomal microarray testing.

| Patient number | Age (year) | Gender | Platform (k) | Start[a] | End[a] | Additional aberration | FISH probe | Mosaic ratio[b] (%) | References |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 14 | F | 180 | 834,101 | 1,770,669 | Interstitial | RP11–425E15 | | |
| 2 | 9 | M | 44 | 1 | 1,820,584 | der(1)t(Y;1), idic(Y) | | | |
| 3 | 6 | F | 180 | 1 | 2,186,829 | | | | |
| 4 | 1 | F | 60 | 1 | 2,239,497 | | | | |
| 5 | 3 | F | 44 | 1 | 2,281,699 | | | | |
| 6 | 5 | F | 60 | 1 | 2,553,982 | | | | |
| 7 | 2 | M | 60 | 1 | 2,553,982 | | | | |
| 8 | 5 | F | 44 | 1 | 3,044,953 | Mosaicism | RP11–82D16 | 70 | Shimada et al. [17] |
| 9 | 13 | F | 44 | 1 | 3,102,718 | | | | Okamoto et al. [14] |
| 10 | 18 | F | 44 | 1 | 3,102,718 | der(1)t(1;7) | | | |
| 11 | 17 | F | 60 | 1 | 3,138,565 | der(1)t(1;8) | | | |
| 12 | 8 | F | 60 | 1 | 3,265,702 | | | | |
| 13 | 11 | F | 244 | 1 | 3,408,152 | | | | |
| 14 | 5 | M | 60 | 1,786,789 | 3,472,907 | Interstitial | | | |
| 15 | 2 | F | 180 | 1 | 3,564,328 | | | | |
| 16 | 13 | M | 60 | 1 | 3,582,084 | | | | |
| 17 | 4 | F | 44 | 1 | 3,607,275 | | | | |
| 18 | 2 | F | 60 | 1 | 3,660,110 | | | | |
| 19 | 3 | F | 60 | 1 | 3,769,006 | Mosaicism | CTD-3209F18 | 28 | |
| 20 | 3 | M | 44 | 1 | 4,070,842 | der(1)t(1;13) | | | |
| 21 | 17 | F | 44 | 1 | 4,481,324 | Mosaicism | RP11–82D16 | 77 | Shimada et al. [17] |
| 22 | 2 | F | 180 | 1 | 4,703,581 | | | | |
| 23 | 6 | M | 60 | 1 | 4,779,157 | | | | |
| 24 | 3 | F | 60 | 1 | 4,843,370 | | | | |
| 25 | 6 | F | 44 | 1 | 4,843,718 | | | | |
| 26 | 2 | M | 60 | 1 | 5,252,985 | | | | |
| 27 | 0 | F | 44 | 1 | 5,252,985 | | | | |
| 28 | 25 | F | 44 | 1 | 5,411,803 | der(1)t(Y;1) | | | Hiraki et al. [15] |
| 29 | 3 | F | 44 | 1 | 6,128,223 | | | | |
| 30 | 3 | F | 60 | 1 | 6,282,562 | | | | |
| 31 | 1 | F | 60 | 1 | 6,282,562 | | | | |
| 32 | 3 | M | 60 | 1 | 6,882,431 | | | | |
| 33 | 7 | M | 60 | 1 | 7,035,075 | | | | |
| 34 | 1 | F | 60 | 1 | 7,187,535 | der(1)t(1;4) | | | |
| 35 | 10 | M | 60 | 1 | 7,392,688 | | | | |
| 36 | 8 | M | 60 | 1 | 7,581,058 | | | | |
| 37 | 3 | F | 44 | 1 | 8,077,959 | | | | |
| 38 | 2 | F | 60 | 1 | 8,104,671 | | | | |
| 39 | 3 | M | 44 | 1 | 8,104,671 | | | | |
| 40 | 4 | M | 44 | 1 | 8,181,042 | | | | |
| 41 | 5 | F | 44 | 1 | 8,181,042 | | | | |
| 42 | 1 | F | 60 | 1 | 8,427,633 | | | | |
| 43 | 3 | M | 60 | 1 | 9,180,975 | der(1)(1;4) | | | Saito et al. [16] |
| 44 | 5 | F | 60 | 1 | 9,251,936 | | | | |
| 45 | 4 | M | 60 | 1 | 9,953,030 | | | | |

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