Virus Genes (2014) 48:448–456
DOI 10.1007/s11262-014-1036-1

Susceptibility of muridae cell lines to ecotropic murine leukemia virus and the cationic amino acid transporter 1 viral receptor sequences: implications for evolution of the viral receptor

Katsura Kakoki · Akio Shinohara · Mai Izumida · Yosuke Koizumi · Eri Honda · Goro Kato · Tsukasa Igawa · Hideki Sakai · Hideki Hayashi · Toshifumi Matsuyama · Tetsuo Morita · Chihiro Koshimoto · Yoshinao Kubo

Received: 22 October 2013 / Accepted: 9 January 2014 / Published online: 28 January 2014

Abstract Ecotropic murine leukemia viruses (Eco-MLVs) infect mouse and rat, but not other mammalian cells, and gain access for infection through binding the cationic amino acid transporter 1 (CAT1). Glycosylation of the rat and hamster CAT1s inhibits Eco-MLV infection, and treatment of rat and hamster cells with a glycosylation inhibitor, tunicamycin, enhances Eco-MLV infection. Although the mouse CAT1 is also glycosylated, it does not inhibit Eco-MLV infection. Comparison of amino acid sequences between the rat and mouse CAT1s shows amino acid insertions in the rat protein near the Eco-MLV-binding motif. In addition to the insertion present in the rat CAT1, the hamster CAT1 has additional amino acid insertions. In contrast, tunicamycin treatment of mink and human cells does not elevate the infection, because their CAT1s do not have the Eco-MLV-binding motif. To define the evolutionary pathway of the Eco-MLV receptor, we analyzed CAT1 sequences and susceptibility to Eco-MLV infection of other several murinae animals, including the southern vole (*Microtus rossiaemeridionalis*), large Japanese field mouse (*Apodemus speciosus*), and Eurasian harvest mouse (*Micromys minutus*). Eco-MLV infection was enhanced by tunicamycin in these cells, and their CAT1 sequences have the insertions like the hamster CAT1. Phylogenetic analysis of mammalian CAT1s suggested that the ancestral CAT1 does not have the Eco-MLV-binding motif, like the human CAT1, and the mouse CAT1 is thought to be generated by the amino acid deletions in the third extracellular loop of CAT1.

Keywords Ecotropic murine leukemia virus · CAT1 · Glycosylation · Evolution

K. Kakoki · M. Izumida · Y. Koizumi · E. Honda · H. Hayashi · T. Matsuyama · Y. Kubo (✉)
Division of Cytokine Signaling, Graduate School of Biomedical Sciences, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852-8523, Japan
e-mail: yoshinao@nagasaki-u.ac.jp

K. Kakoki · T. Igawa · H. Sakai
Department of Urology, Graduate School of Biomedical Sciences, Nagasaki University, Nagasaki 852-8523, Japan

A. Shinohara · G. Kato · C. Koshimoto
Division of Biotechnology, Department of Bio-resources, Frontier Science Research Center, University of Miyazaki, Miyazaki 889-1692, Japan

T. Morita
Department of Plant and Animal Science, Faculty of Agriculture, University of Miyazaki, Miyazaki 889-2192, Japan

Y. Kubo
Department of AIDS Research, Institute of Tropical Medicine, G-COE, Nagasaki University, Nagasaki 852-8523, Japan

Introduction

A change in the cell surface receptor for a virus is one of a host defense mechanism against virus infection; for example, the C-terminally truncated CCR5 variant is known to confer resistance to human immunodeficiency virus (HIV) infection [1]. We have analyzed the viral receptors for ecotropic murine leukemia viruses (Eco-MLVs) in mouse (*Mus musculus*), rat (*Ratus norvegicus*), and *Mus dunni* cells as a model of receptor evolution that confers resistance to a virus infection [2–4].

Eco-MLVs can infect mouse and rat cells, and recognize the multi-membrane-spanning cationic amino acid transporter 1 (CAT1) as the receptor for infection [5]. Eco-MLV binds the YGE or HGE motif in the third extracellular loop of the CAT1 [6, 7]; the CAT1 has two N-linked

glycosylation sites near the Eco-MLV-binding motif. Nucleotide sequences near the virus-binding motif are highly diversified among mouse, rat, hamster, mink, and human, suggesting that the region is under selective pressure.

Rat cells are much less susceptible to Eco-MLV infection than mouse cells, and hamster cells are completely resistant to infection. Treatment of rat and hamster cells with tunicamycin, an N-linked glycosylation inhibitor, enhances susceptibility to Eco-MLV infection [8, 9]. Furthermore, an amino acid substitution at the glycosylation site of the rat CAT1 increases susceptibility to Eco-MLV infection [2]. These results indicate that N-linked glycosylation of the rat and hamster CAT1 proteins inhibits Eco-MLV infection. Although the mouse CAT1 is also glycosylated at the same amino acid residues as the rat and hamster CAT1s, it does not affect Eco-MLV infection [2]; rat and hamster CAT1 proteins have three- and six-amino acid insertions near the viral-binding domain of the protein compared to the mouse CAT1. We have previously reported that a deletion of the amino acid insertion in the rat CAT1 confers increased susceptibility, and abrogates the glycosylation-mediated inhibition of Eco-MLV infection, indicating that the amino acid insertion in the rat CAT1 is the determinant for the glycosylation-dependent infection inhibition [3]. The longer insertion present in the hamster CAT1 compared to the rat protein may confer hamster cells a complete resistance to Eco-MLV infection. In addition, glycosylation of the *Mus dunni* CAT1 also inhibits Eco-MLV infection as a result of a one-amino acid insertion in the YGE virus-binding motif [4].

To confirm the pathway of evolution for mammalian CAT1s, we established immortalized cell lines from several murinae animals, and then determined their susceptibility to Eco-MLV infection and their CAT1 sequences. We showed that the CAT1 sequences of the southern vole, large Japanese field mouse, and Eurasian harvest mouse were shown to have amino acid insertions similar to the hamster CAT1. Phylogenetic analysis of mammalian CAT1 sequences revealed that the CAT1 ancestor is the human-type CAT1, and evolved to the mouse-type CAT1 by deletion rather than by insertion. This study reviews the evolutionary pathway of the ecotropic MLV receptor, CAT1, in relation to the viral infection.

Materials and methods

Animals

The southern vole (*Microtus rossiaemeridionalis*) and steppe lemming (*Lagurus lagurus*) were obtained from the closed colony maintained at the Frontier Science Research Center, University of Miyazaki. The Mongolian gerbil (*Meriones unguiculatus*) was obtained from the closed colony maintained at the Institute of Tropical Medicine, Nagasaki University. A wild large Japanese field mouse (*Apodemus speciosus*) and a Eurasian harvest mouse (*Micromys minutus*) were captured in Kiyotake, Miyazaki City, Miyazaki Prefecture, Japan, with the approval of the prefectural governor (No. 24940-2696). This animal study is approved by the Ethics Committee of Nagasaki University (No. 0812080723), and the Committee for the Ethics on Animal Experiments at the University of Miyazaki (No. 2008-505).

Cells

Mouse NIH3T3, rat F10, and human TELCeB6 [10] cells were cultured in Dulbecco's modified Eagle's medium (D-MEM) supplemented with 8 % fetal bovine serum (FBS). Kidney grafts of *M. minutus*, *M. rossiaemeridionalis,* and muscle grafts of *A. speciosus* were isolated and were treated with trypsin to separate cells. The cells were cultured with D-MEM containing 20 % FBS for more than one year. Cells from *M. rossiaemeridionalis* and *A. speciosus* were passed by 1/6 dilution every 3 days. Cells from *M. minutus* were passed by 1/2 dilution every 6 days.

Expression plasmids

An expression plasmid of the ecotropic Friend MLV Env has been already described [11]. A VSV-G expression plasmid was obtained from Dr. L. Chang through the AIDS Research and Reference Reagent Program, NIAID, NIH, USA [12].

Transduction assay

TELCeB6 cells [10] were transfected with an expression plasmid containing the Friend MLV envelope protein using the Fugene transfection reagent (Promega). Since the expression plasmid also encodes the neomycin resistance gene, the transfected cells were selected by geneticin (Invitrogen). Culture supernatants of the geneticin-resistant cell pool were inoculated into target cells in the presence of Polybrene (4 μg/ml) (Sigma–Aldrich). To construct a VSV-pseudotyped MLV vector, TELCeB6 cells were transiently transfected with a VSV-G expression plasmid and their culture supernatants were collected 2 days after transfection. The culture supernatants were then inoculated into target cells. To estimate transduction titer, the inoculated cells were stained with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) (Wako) 2 days after the inoculation and then blue cells were counted.

Isolation of CAT1 sequences

Total RNA samples were prepared from cells using Isogen (Invitrogen). cDNAs were synthesized by reverse transcriptase (TaKaRa) and a fragment containing the third extracellular loop of CAT1 and its upstream region was amplified by PCR (TaKaRa) using the cDNA as template. Nucleotide sequences of the PCR primers are AAC CTG ATT CTC TCC TAC ATC and GTG GTG GCG ATG CAG TCA AAG. The PCR products were cloned into pTarget vector (Promega) and nucleotide sequences of the insert DNAs were determined (Applied Biosystems). The primers were synthesized by Genenet Co., LTD. Accession numbers of CAT1s from A. speciosus, *M. rossiaemeridionalis,* and *M. minutus* are AB839945, AB839946, and AB839947, respectively.

Alignments and phylogenetic analysis

Rodent CAT1 gene sequences were compared with other mammalian CAT1 gene sequences obtained from the DNA database: laboratory rat (*Rattus norvegicus*; AB066224) [2], laboratory mouse (*Mus musculus*; M26687) [5], human (*Homo sapiens*; X59155) [7], pig (*Sus scrofa*; AY371320) [13], domestic dog (*Canis lupus familiaris*; XM_854224) [14], American mink (*Neovison vison*; U49796) [15], domestic cat (*Felis catus*; XM_003980275) [16], chimpanzee (*Pan troglodytes*; XM_001139004), horse (*Equus caballus*; XM_001492839) [17], cattle (*Bos taurus*; NM_001135792) [18], giant panda (*Ailuropoda melanoleuca*; XM_002914759) [19], African elephant (*Loxodonta africana*; XM_0034 14018), bonobo (*Pan paniscus*; XM_003818324) [20], Syrian hamster (*Mesocricetus auratus*; U26454), Chinese hamster (*Cricetulus griseus*; U49797), orangutan (*Pongo abelii*; XM_002824135) [21], small-eared galago (*Otolemur garnettii*; XM_003797609), Northern white-cheeked gibbon (*Nomascus leucogenys*; XM_003270277), rabbit (*Oryctolagus cuniculus*; XM_002721425), lesser Egyptian jerboa (*Jaculus jaculus*; XM_004659984), naked mole rat (*Heterocephalus glaber*; XM_004854792), and degu (*Octodon degus*; XM_004631056).

The obtained three rodent CAT1 gene sequences were compared with other mammalian CAT1 gene sequences obtained from the DNA database. All the sequences were once translated into amino acids and then aligned using MUSCLE [22] implemented in MEGA ver 5.1 [23]. These aligned amino acid sequences were reversely translated into nucleotide sequences, and used for the analyses.

For phylogenetic analyses, we employed the CAT2 sequences of human (D29990) [24] and mouse (L03290) [25] for the outgroup. Therefore, we realigned all the sequences with these CAT2 sequences following the same procedure described above. A phylogenetic tree was constructed by Bayesian method. The dataset was divided into three partitions with codon position (1st, 2nd, and 3rd), and optimum substitution models for each partition were selected by Kakusan 4 [26] based on the Bayesian information criterion; general time reversible (GTR) [27] with gamma distribution (+G), GTR+G, and HKY85 [28] +G models were selected for the 1st, 2nd, and 3rd positions, respectively. The Bayesian analysis was conducted using MrBayes v3.2.1 [29] with 3 million generations of two independent runs of four Markov chains. We sampled one tree every 100 generations and calculated a consensus topology with discarding the first 25 % of trees. The final average standard deviation of split frequencies of the Bayesian analysis was 0.017942, and all average effective sample sizes were more than 200.

Statistical analysis

Differences between two groups of data were determined by the Student's *t* test. The statistical significance was set at $P < 0.05$ for all the tests.

Results

Susceptibility of rodent cells to Eco-MLV infection

Immortalized cell lines were established from the inbred southern vole (*Microtus rossiaemeridionalis*), wild large Japanese field mouse (*Apodemus speciosus*), and wild Eurasian harvest mouse (*Micromys minutus*) to assess their susceptibility to Eco-MLV infection. Cells were inoculated with the Friend MLV Env protein-carrying MLV vector, and transduction titers were measured. Infected cells were detected in the *A. speciosus* and *M. rossiaemeridionalis* cells; we observed the transduction titers for these cells to be 1/100–1/1000 times lower than those of mouse NIH3T3 cells (*M. musculus*), similar to that found on rat F10 cells (*R. norvegicus*) (Fig. 1a). In contrast, infected cells were not detected from *M. minutus*, showing that these cells are resistant to Eco-MLV infection.

To determine whether N-linked glycosylation inhibits the Eco-MLV vector infection in these rodent cells, the cells were pretreated with tunicamycin (100 μg/ml) for 24 h, and then were inoculated with the Eco-MLV vector. In the *A. speciosus, M. minutus,* and *M. rossiaemeridionalis* cells, tunicamycin treatment enhanced Eco-MLV vector infection, as in the rat F10 cells (Fig. 1b), showing that glycosylation inhibits the Eco-MLV infection in these cells. In contrast, we have already reported that tunicamycin treatment of mouse NIH3T3 cells does not affect the Eco-MLV vector infection [2, 3]. However, infection by VSV-G-pseudotyped MLV vector was not affected by the

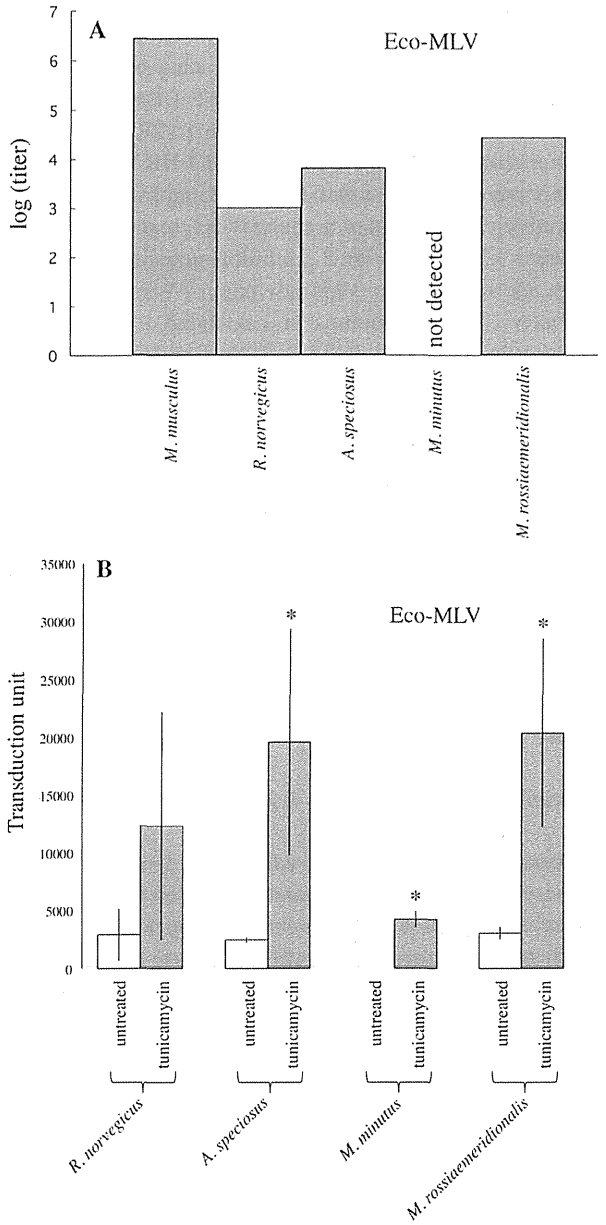

Fig. 1 Susceptibility of rodent cells to Eco-MLV infection. Rodent cells were inoculated with the Eco-MLV (**a** and **b**). Cells were treated or untreated with tunicamycin for 24 h and then were inoculated with the viral vector. These experiments were repeated three times. The results are shown as the average titers ± SD. *Asterisks* indicate statistically significant differences compared to the titer on the untreated cells

tunicamycin treatment of these cells (data not shown), suggesting that the glycosylation-mediated inhibition of MLV infection is dependent on the virus envelope protein. These data indicate that glycosylation of CAT1 proteins of *A. speciosus, M. minutus,* and *M. rossiaemeridionalis* inhibits Eco-MLV infection, suggesting that these CAT1 sequences have the amino acid insertion similar to the rat and hamster CAT1.

Nucleotide sequences of the CAT1 genes of rodent cells

DNA fragments containing the third extracellular loop and its upstream region of the CAT1 gene were isolated by RT-PCR from *M. rossiaemeridionalis, A. speciosus, and M. minutus* cells, and their nucleotide sequences were determined. Amino acid sequences of the third extracellular loop and its upstream region from the various mammals are shown in Fig. 2a, b, respectively. The CAT1 sequences of *M. rossiaemeridionalis, A. speciosus,* and *M. minutus* contain the amino acid insertions and the YGE or HGE Eco-MLV-binding motif in the third extracellular loop, as found in the hamster CAT1. Interestingly, the CAT1 sequences from rabbit (*Oryctolagus cuniculus*) and lesser Egyptian jerboa (*Jaculus jaculus*) have divergent types of deletions in the third extracellular loop.

When nucleotide sequences were compared among rodents including *M. musculus, R. norvegicus, M. minumus, A. speciosus, M. rossiaemeridionalis*, Chinese hamster (*C. griseus*), Syrian hamster (*M. auratus*), naked mole rat (*H. glaber*), degu (*O. degus*), rabbit (*O. cuniculus*), and lesser Egyptian jerboa (*J. jaculus*), the third extracellular loops (Fig. 3a) (76.4 ± 6.8 %) were less homologous than their upstream regions (Fig. 3b) (89.1 ± 4.8 %) ($P = 9.49 \times 10^{-23}$). Furthermore, the changes in the first and second nucleotides of codons in the CAT1 third extracellular loops (ECL3s) of rodents were more dominant than those in the upstream regions (Table 1), and nonsynonymous mutations in the ECL3s were more abundant than those in the upstream regions, compared to the mouse CAT1 ($P = 8.12 \times 10^{-11}$). In contrast, the amino acid sequences of the extracellular loops of the CAT1s among cat, dog, pig, horse, cattle, giant panda, elephant, small-eared galago, northern white-cheeked gibbon, Sumatran orangutan, bonobo, chimpanzee, and human are more similar than those among the rodents (Fig. 3c) (86.8 ± 7.2 %) ($P = 2.93 \times 10^{-14}$). These results suggest that the third extracellular region is under stronger selective pressure in rodents than in higher mammals.

Additionally, immortalized cells were established from inbred steppe lemming (*Lagurus lagurus*) and Mongolian gerbil (*Meriones unguiculatus*). CAT1 sequences could not be amplified from these cells by RT-PCR (data not shown). Consistently, when these cells were inoculated with the Eco-MLV vector, LacZ-expressing cells were not detected even in the presence of tunicamycin, although VSV-G-pseudotyped vector could transduce the cells. The *L. lagurus* and *M. unguiculatus* cells might be resistant due to the lack of susceptible CAT1 expression.

Fig. 2 Amino acid sequences of CAT1s. The amino acid sequences of the third extracellular loops (**a**) and the upstream regions (**b**) of CAT1s are indicated. *Bars* and *dots* indicate identical amino acids and deletions, respectively. N-linked glycosylation sites are *underlined*. The Eco-MLV binding motif is *double-underlined*

Fig. 3 Comparison of nucleotide sequences of CAT1s. Nucleotide sequences of the third extracellular loops (**a**) and the upstream regions (**b**) of CAT1s from indicated animals. Nucleotide sequences of the third extracellular loops (**a**) and their upstream regions (**b**) of rodent CAT1s are compared. Nucleotide sequences of the third extracellular loops of CAT1s from the higher animals are compared (**c**)

A

	M. musculus	*R.norvegicus*	*M. minutus*	*A. speciosus*	*M. rossiaemeridionalis*	*C. griseus*	*M. auratus*	*H. glaber*	*O. degus*	*O. cuniculus*	*J. jaculus*
M. musculus	100	74	76	76	71	74	74	70	72	64	66
R.norvegicus		100	82	74	82	82	82	75	74	71	69
M. minutus			100	87	76	87	82	80	79	72	69
A. speciosus				100	76	84	79	80	80	70	72
M. rossiaemeridionalis					100	89	94	81	79	68	68
C. griseus						100	94	82	80	72	72
M. auratus							100	78	77	68	68
H. glaber								100	89	72	73
O. degus									100	73	72
O. cuniculus										100	74
J. jaculus											100

B

	M. musculus	*R.norvegicus*	*M. minutus*	*A. speciosus*	*M. rossiaemeridionalis*	*C. griseus*	*M. auratus*	*H. glaber*	*O. degus*	*O. cuniculus*	*J. jaculus*
M. musculus	100	98	96	98	88	92	90	88	86	85	88
R.norvegicus		100	98	100	90	94	92	85	84	83	87
M. minutus			100	98	90	94	92	86	86	84	89
A. speciosus				100	90	94	92	84	83	84	89
M. rossiaemeridionalis					100	96	96	87	87	83	85
C. griseus						100	98	85	88	85	89
M. auratus							100	84	86	83	86
H. glaber								100	92	87	85
O. degus									100	87	89
O. cuniculus										100	86
J. jaculus											100

C

	F. catus	*C. lupus*	*S. scrofa*	*E. caballus*	*B. taurus*	*A. melanoleuca*	*L. africana*	*O. garnettii*	*N. leucogenys*	*P. abelii*	*P. paniscus*	*P. troglodytes*	*H. sapiens*
F. catus	100	79	87	79	87	82	82	82	82	82	82	82	82
C. lupus		100	79	77	82	85	82	77	79	79	79	79	79
S. scrofa			100	90	95	79	82	92	90	90	90	90	90
E. caballus				100	92	77	90	95	85	85	85	85	85
B. taurus					100	85	87	95	92	92	92	92	92
A. melanoleuca						100	79	79	77	77	77	77	77
L. africana							100	87	87	87	87	87	87
O. garnettii								100	90	90	90	90	90
N. leucogenys									100	100	100	100	100
P. abelii										100	100	100	100
P. paniscus											100	100	100
P. troglodytes												100	100
H. sapiens													100

Table 1 Changes of the first, second, and third bases of codons in mammalian CAT1s compared to mouse CAT1s

Species	Ratios of changes in upstream of ECL3 (%)			Ratios of changes in ECL3 (%)		
	First	Second	Third	First	Second	Third
R. norvegicus	0.8	0	6.9	4.0	3.4	5.1
M. minutus	0.8	0	0.5	5.1	4.5	6.2
A. speciosus	1.1	0	8.0	4.5	4.5	7.3
C. griseus	1.9	1.1	8.4	5.6	4.5	11.3
M. auratus	1.3	0.4	8.0	5.1	5.1	9.6
M. rossiaemeridionalis	3.4	0.4	9.6	5.6	5.1	11.3
H. glaber	2.7	0	9.2	6.8	6.8	16.4
O. Degus	2.7	0.4	10.7	7.3	6.8	13.6
O. cuniculus	3.8	1.1	10.0	7.9	9.0	18.6
J. jaculus	2.3	1.1	8.4	9.6	9.8	16.9

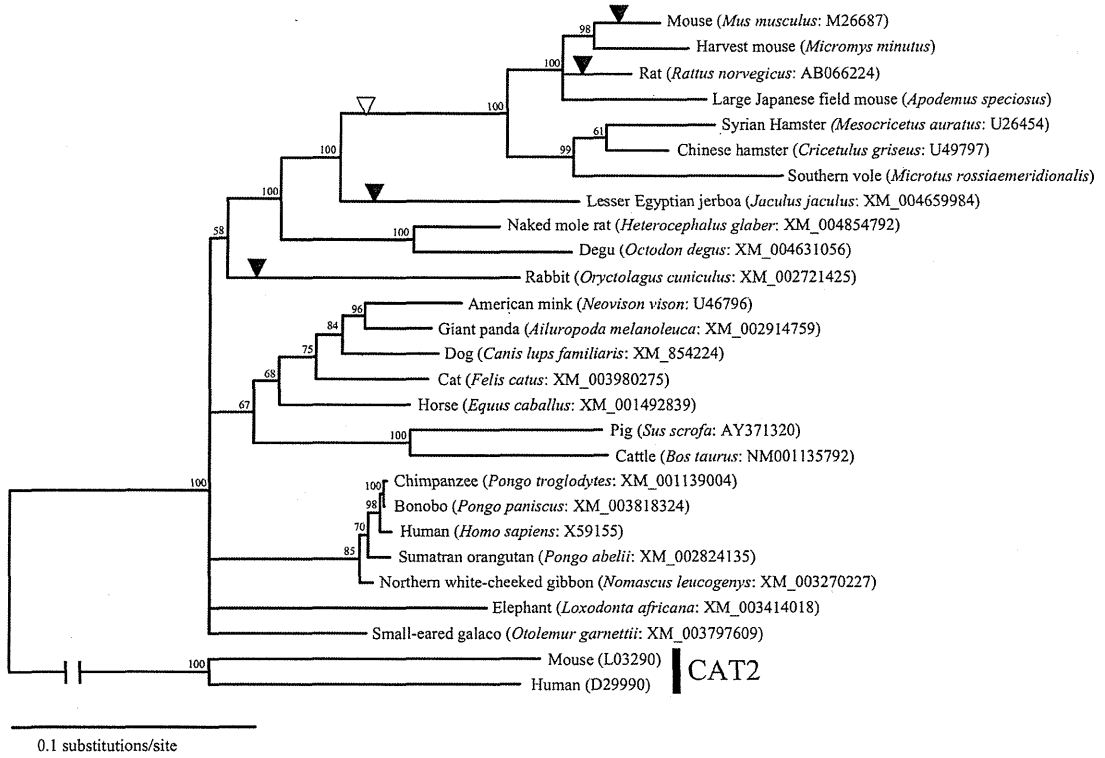

Fig. 4 Phylogenetic tree of the mammalian CAT1s. *Closed arrow heads* indicate the time points when amino acid deletions in the third extracellular loops of CAT1s occurred. An *open arrow head* shows the time point when the Eco-MLV-binding motif was obtained

Phylogenetic analysis of the ecotropic receptors

The phylogenetic tree of the CAT1s from the various mammals was constructed and is shown in Fig. 4. Rodents including mouse, rat, hamster, *A. speciosus, M. minutus*, and *M. rossiaemeridionalis* belong to one group. Human and primates belong to another group. The phylogenetic tree is consistent with the standard classification of mammals. By the phylogenetic tree, the Eco-MLV-binding motif could be obtained before mouse, rat, and hamster appeared (open arrow head in Fig. 4), and the amino acid deletions in the third extracellular loops of mouse, rat, rabbit, and lesser Egyptian jerboa CAT1s could occur independently (closed arrow heads in Fig. 4). This phylogenetic analysis also suggests that the ancestor CAT1 contain the amino acid insertion as present in the human CAT1s, and thereafter the mouse- and rat-type

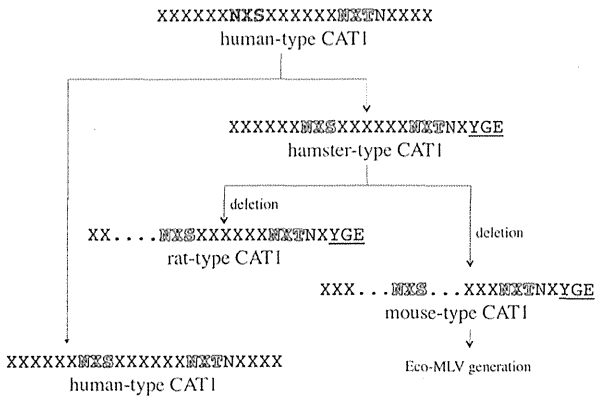

Fig. 5 Evolutionary pathway of CAT1 predicted by this study. *Block letters* show glycosylation sites. *Dots* show amino acid deletions. The Eco-MLV-binding motifs are *underlined*

CAT1s could be generated from the hamster-type CAT1 by amino acid deletion (Fig. 5).

Discussion

This study provides a putative evolution pathway of the mammalian CAT1s as follows (Fig. 5): the ancestor of the Eco-MLV receptor in mammals might not have the Eco-MLV-binding motif, like the human CAT1. Since Eco-MLV cannot infect the human CAT1, Eco-MLVs should not circulate at that time. The ancestral human-type CAT1 might be converted to the hamster-type CAT1, acquiring the virus-binding motif (open arrow head in Fig. 4). Since the hamster-type CAT1 is much less susceptible to Eco-MLV infection than the mouse CAT1, Eco-MLV should not spread at that time. The mouse CAT1 might be generated from the hamster-type CAT1 through amino acid deletion (closed arrow in Fig. 4). Since the mouse CAT1 is fully susceptible to Eco-MLV infection, Eco-MLV could circulate after the deletion occurred. Consistent with this speculation, it has been reported that many wild mice contain endogenous polytropic and xenotropic MLV sequences in their genomes but wild mice carrying the endogenous ecotropic MLV sequence are less numerous [30], suggesting that the ecotropic MLV appeared later than the polytropic and xenotropic viruses. Therefore, it is unlikely that Eco-MLV was derived from a virus which is related to Eco-MLV and efficiently interacts with the hamster-type CAT1 in the ancestor species of the *Mus* genus. Since the third extracellular loops of *Mus* subgenus animals, including *M. dunni*, *M. spicilegus*, and *M. minutoides*, have amino acid deletions as seen in the *M. musculus* CAT1 [31, 32], the deletion is thought to have occured before the *Mus* subgenus appeared.

The homology of the CAT1 third extracellular loop among the rodents is relatively lower than among the higher mammals, suggesting that the regions are under selective pressure in rodents. Since phylogenetic analysis of the mammalian CAT1s provided a possibility that Eco-MLV appeared after the *Mus* subgenus was generated, the selective pressure might not be the Eco-MLV infection itself. The CAT1 containing the amino acid deletion might be advantageous in the *Mus* subgenus, and eventually the deletion might permit the appearance of EcoMLVs. The observation that mouse, rat, rabbit, and lesser Egyptian jerboa CAT1s independently have amino acid deletions in the third extracellular loop suggests that the third extracellular loop of CAT1 is disadvantageous for rodents.

Since the one-amino acid insertion in the *Mus dunni* CAT1 is not found in the CAT1s of other *Mus* animals, it is thought that the one-amino acid insertion occurred to inhibit infection by Eco-MLV or other CAT1-recognizing viruses after the *Mus* subgenus appeared. CAT1-recognizing virus(s) might be widely spread among *M. dunni* population. To resolve this issue, further study is required.

Acknowledgments The VSV-G expression plasmid was kindly obtained from Dr. L. Chang through the AIDS Research and Reference Reagent Program, NIAID, NIH, USA. TELCeB6 cells were kindly provided by Dr. F. Cossett. We thank Ms. F. Tsujita, Ms. Y. Kobayashi, and Ms. M. Haraguchi for assistance. This study was partially supported by the Japan Society for the Promotion of Science.

References

1. Y. Huang, W.A. Paxton, S.M. Wolinsky, A.U. Neumann, L. Zhang, T. He, S. Kang, D. Ceradini, Z. Jin, K. Yazdanbakhsh, K. Kunstman, D. Erickson, E. Dragon, N.R. Landau, J. Phair, D.D. Ho, R.A. Koup, Nat. Med. **2**, 1240–1243 (1996)
2. Y. Kubo, T. Ono, M. Ogura, A. Ishimoto, H. Amanuma, Virology **303**, 338–344 (2002)
3. Y. Kubo, A. Ishimoto, T. Ono, H. Yoshii, C. Tominaga, C. Mitani, H. Amanuma, N. Yamamoto, Virology **330**, 82–91 (2004)
4. H. Yoshii, H. Kamiyama, H. Amanuma, K. Oishi, N. Yamamoto, Y. Kubo, J. Gen. Virol. **89**, 297–305 (2008)
5. L.M. Albritton, L. Tseng, D. Scadden, J.M. Cunningham, Cell **57**, 659–666 (1989)
6. L.M. Albritton, J.W. Kim, L. Tseng, J.M. Cunningham, J. Virol. **67**, 2091–2096 (1993)
7. T. Yoshimoto, E. Yoshimoto, D. Meruelo, J. Virol. **67**, 1310–1314 (1993)
8. M.V. Eiden, K. Farrell, C.A. Wilson, J. Virol. **67**, 4056–4061 (1994)
9. N. Tavoloni, A. Rudenholz, Virology **229**, 49–56 (1997)
10. F.L. Cosset, Y. Takeuchi, J.L. Battini, R.A. Weiss, M.K. Collins, J. Virol. **69**, 7430–7436 (1995)
11. Y. Kubo, A. Ishimoto, H. Amanuma, J. Virol. **77**, 7510–7516 (2003)
12. L.J. Chang, V. Urlacher, T. Iwakura, Y. Cui, J. Zucali, Gene Ther. **6**, 715–728 (1999)
13. Z. Cui, S. Zharikov, S.L. Via, S.I. Anderson, A.S. Law, A.L. Archibald, E.R. Block, Genomics **85**, 352–359 (2005)

14. K. Lindblad-Toh, C.M. Wade, T.S. Mikkelsen, E.K. Karlsson, D.B. Jaffe, M. Kamal, M. Clamp, J.L. Chang et al., Nature **438**, 803–819 (2005)
15. H. Wang, E. Klamo, S.E. Kuhmann, S.L. Kozak, M.P. Kavanaugh, D. Kabat, J. Virol. **70**, 6884–6891 (1996)
16. J.U. Pontius, J.C. Mullikin, D.R. Smith, Agencourt Sequencing team et al., Genome Res. **17**, 1675–1689 (2007)
17. C.M. Wade, E. Giulotto, S. Sigurdsson, M. Zoli, S. Gnerre, F. Imsland, T.L. Lear, D.L. Adelson, E. Bailey et al., Science **326**, 865–867 (2009)
18. A.V. Zimin, A.L. Delcher, L. Florea, D.R. Kelley, M.C. Schatz, D. Puiu, F. Hanrahan, G. Pertea, C.P. van Tassell, T.S. Sonstegard, G. Marcais, M. Roberts, P. Subramanian, J.A. Yorke, S.L. Salzberg, Genome Biol. **10**, R42 (2009)
19. R. Li, W. Fan, G. Tian, H. Zhu, L. He, J. Cai, Q. Huang, Q. Cai, B. Li, Y. Bai, Z. Zhang, Y. Zhang, W. Wang et al., Nature **463**, 311–317 (2010)
20. K. Prufer, K. Munch, I. Hellmann, K. Akagi, J.R. Miller, B. Walenz, S. Koren, G. Sutton, C. Kodira, R. Winer et al., Nature **486**, 527–531 (2012)
21. D.P. Locke, L.W. Hillier, W.C. Warren, K.C. Worley, L.V. Nazareth, D.M. Muzny, S.P. Yang, Z. Wang, A.T. Chinwalla et al., Nature **469**, 529–533 (2011)
22. R.C. Edgar, Nucleic Acid Res. **32**, 1792–1797 (2004)
23. K. Tamura, D. Peterson, N. Peterson, G. Stecher, M. Nei, S. Kumar, Mol. Biol. Evol. **28**, 2731–2739 (2011)
24. R. Hoshide, Y. Ikeda, S. Karashima, T. Matsuura, S. Komaki, T. Kishino, N. Niikawa, F. Endo, I. Matsuda, Genomics **38**, 174–178 (1996)
25. E.I. Closs, L.M. Albritton, J.W. Kim, J.M. Cunningham, J. Biol. Chem. **268**, 7538–7544 (1993)
26. A.S. Tanabe, Mol. Ecol. Resour. **11**, 914–921 (2011)
27. S. Tavaré, Some probabilistic and statistical problems in the analysis of DNA sequences, in *Some mathematical questions in biology–DNA sequence analysis*, ed. by R.M. Miura (American Mathematical Society, Providence, RI, 1986), pp. 57–86
28. M. Hasegawa, H. Kishino, T. Yano, J. Mol. Evol. **22**, 160–174 (1985)
29. F. Ronquist et al., Syst. Biol. **61**, 539–542 (2012)
30. C.A. Kozak, R.R. O'Neill, J. Virol. **61**, 3082–3088 (1987)
31. Y.T. Jung, C.A. Kozak, J. Virol. **77**, 5065–5072 (2003)
32. Y. Yan, C.A. Kozak, J. Virol. **82**, 6120–6129 (2008)

ELSEVIER

Brain & Development 34 (2012) 337–343

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

www.elsevier.com/locate/braindev

Original article

Epidemiology of acute encephalopathy in Japan, with emphasis on the association of viruses and syndromes

Ai Hoshino [a], Makiko Saitoh [a,*], Akira Oka [b], Akihisa Okumura [c], Masaya Kubota [d], Yoshiaki Saito [e], Jun-ichi Takanashi [f], Shinichi Hirose [g], Takanori Yamagata [h], Hideo Yamanouchi [i], Masashi Mizuguchi [a]

[a] *Department of Developmental Medical Sciences, Graduate School of Medicine, The University of Tokyo, Japan*
[b] *Department of Pediatrics, Kyorin University School of Medicine, Mitaka, Japan*
[c] *Department of Pediatrics, Juntendo University School of Medicine, Tokyo, Japan*
[d] *Division of Neurology, National Center for Child Health and Development, Tokyo, Japan*
[e] *Department of Child Neurology, National Center of Neurology and Psychiatry, Kodaira, Japan*
[f] *Department of Pediatrics, Kameda Medical Center, Kamogawa, Japan*
[g] *Department of Pediatrics, School of Medicine, Fukuoka University, Fukuoka, Japan*
[h] *Department of Pediatrics, Jichi Medical University, Shimotsuke, Japan*
[i] *Department of Pediatrics, Saitama Medical University, Saitama, Japan*

Received 19 April 2011; received in revised form 27 July 2011; accepted 30 July 2011

Abstract

A research committee supported by the Japanese government conducted a nationwide survey on the epidemiology of acute encephalopathy in Japan using a questionnaire. A total of 983 cases reportedly had acute encephalopathy during the past 3 years, 2007–2010. Among the pathogens of the preceding infection, influenza virus was the most common, followed by human herpesvirus-6 (HHV-6) and rotavirus. Among syndromes of acute encephalopathy, acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) was the most frequent, followed by clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS), acute necrotizing encephalopathy (ANE) and hemorrhagic shock and encephalopathy syndrome (HSES). Influenza virus was strongly associated with ANE and MERS, HHV-6 with AESD, and rotavirus with MERS. Mortality was high in ANE and HSES, but was low in AESD, MERS and HHV-6-associated encephalopathy. Neurologic sequelae were common in AESD and ANE, but were absent in MERS.

Keywords: Acute encephalopathy; Epidemiology; Acute necrotizing encephalopathy; Acute encephalopathy with biphasic seizures and late reduced diffusion; Clinically mild encephalitis/encephalopathy with a reversible splenial lesion

1. Introduction

Acute encephalopathy is a severe complication of common infections of childhood, such as influenza, exanthem subitum and acute viral gastroenteritis. It usually affects children who have previously been healthy, and often causes death or severe neurological handicaps. There are two classifications of acute encephalopathy [1]. One is based on the pathogen of the preceding infection, such as influenza encephalopathy, human herpesvirus-6 (HHV-6) encephalopathy and rotavirus encephalopathy, whereas the other is based on clinical, laboratory, imaging and pathological findings of encephalopathy. With recent advances in this syndrome classification,

* Corresponding author. Address: Department of Developmental Medical Sciences, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. Tel.: +81 3 5841 3515; fax: +81 3 5841 3628.
E-mail address: makisaito-tky@umin.ac.jp (M. Saitoh).

0387-7604/$ - see front matter © 2011 The Japanese Society of Child Neurology. Published by Elsevier B.V. All rights reserved.
doi:10.1016/j.braindev.2011.07.012

many novel syndromes, such as acute necrotizing encephalopathy (ANE) [2], acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) [3] and clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS) [4], have been established.

At present, epidemiologic data on acute encephalopathy are limited. In the United States, the California Encephalitis Project has collected a large number of cases of central nervous system infection since 1988; however, this study focused primarily on encephalitis, not on encephalopathy [5]. In Japan, several attempts have previously been made to estimate the morbidity and mortality of acute encephalitis/ encephalopathy [6–10]; however, none has used syndrome classification of acute encephalopathy.

In 2010, supported by a grant from the Ministry of Health, Labour and Welfare of Japan, we started the Committee for the Research on the Etiology, Diagnosis and Treatment of Severe and Intractable Acute Encephalopathy, and conducted a nationwide survey of acute encephalopathy in Japan. This study used for the first time both classifications, pathogenic (virological) and syndrome (clinico-pathological) [1], and elucidated the relationship between viruses and syndromes.

2. Material and methods

In this study, we defined acute encephalopathy based on the following criteria: (1) acute onset of impaired consciousness after a preceding infection, and (2) exclusion of well-defined intracerebral inflammation. According to the second criterion, we excluded meningitis/ encephalitis, such as herpes simplex virus (HSV) encephalitis and acute disseminated encephalomyelitis, in which inflammatory pathology is clearly established. On the other hand, we included several conditions in which the distinction between encephalitis and encephalopathy is unclear, such as MERS [4] and acute encephalitis with refractory, repetitive partial seizures (AERRPS) [11]. We also included cases even if the respondent inadvertently failed to answer a single item.

In June 2010, we mailed a questionnaire to the heads of the Department of Pediatrics of 520 hospitals that had been qualified as institutions for training pediatric specialists by the Japanese Pediatric Society. The hospitals included all the pediatric referral centers in Japan, and were distributed all over the country.

The questionnaire items were (1) the number of cases of acute encephalopathy treated by each hospital during the last 3 years (from April 2007 to June 2010), (2) date

Table 1
Diagnostic criteria for three major syndromes.

I. *Acute necrotizing encephalopathy of childhood (ANE)*
1. Acute encephalopathy following a viral febrile disease. Rapid deterioration in the level of consciousness. Convulsions
2. No CSF pleocytosis. Increase in CSF protein commonly observed
3. CT or MRI evidence of symmetric, multifocal brain lesions. Involvement of the bilateral thalami. Lesions also common in the cerebral periventricular white matter, internal capsule, putamen, upper brain stem tegmentum and cerebellar medulla. No involvement of other CNS regions
4. Elevation of serum aminotransferases of variable degrees. No increase in blood ammonia
5. Exclusion of resembling diseases.
A. Differential diagnosis from clinical viewpoints.
Overwhelming bacterial and viral infections, and fulminant hepatits; toxic shock, hemolytic uremic syndrome and other toxin-induced diseases; Reye syndrome, hemorrhagic shock and encephalopathy syndrome, and heat stroke.
B. Differential diagnosis from radiological viewpoints.
Leigh encephalopathy and related mitochondrial cytopathies; glutaric acidemia, methylmalonic acidemia, and infantile bilateral striatal necrosis; Wernicke encephalopathy, and carbon monoxide poisoning; acute disseminated encephalomyelitis, acute hemorrhagic leucoencephalitis, other types of encephalitis and vasculitis; arterial or venous infection, and the effects of severe hypoxia or head trauma

II. *Acute encephalopathy with biphasic seizures and late reduced diffusion (AESD)*
1. Onset with convulsion (status epilepticus convulsivus in most cases) within 24 hours from the onset of fever
2. Subsequent, transient improvement in consciousness
3. Recurrence of convulsions (clustering partial seizures in most cases) on the fourth to sixth day of illness, followed by impairment of consciousness
4. Pathogens of precedent infection: influenza virus and HHV-6, 7 in many cases
5. Variable prognosis: mild to severe psychomotor retardation. Typical cases show impaired speech and voluntariness
6. Normal MRI on the first to second day of illness
7. High signal intensity lesions in the cerebral subcortical white matter on diffusion-weighted images on the third to ninth day of illness. T2-weighted and FLAIR images may show high signal intensities along U-fibers

III. *Clinically mild encephalitis/encephalopathy with a reversible splenial lesion (MERS)*
1. Onset with neuropsychiatric symptoms, such as abnormal speech and/or behavior, and impaired consciousness and convulsion, within one week after the onset of fever
2. Complete recovery without sequelae, mostly within ten days after the onset of neuropsychiatric symptoms
3. High signal intensity lesion in the splenium of corpus callosum, in the acute stage. T1 and T2 signal changes are mild
4. Lesion may involve the entire corpus callosum and the cerebral white matter in a symmetric fashion
5. Lesion disappears within a week, with neither residual signal changes nor atrophy

(year/month) and age at onset of each case, (3) sex, (4) syndrome of acute encephalopathy (e.g. ANE, AESD, MERS and others), (5) pathogen of preceding infection (e.g. influenza virus, HHV-6, unknown and others), and (6) prognosis. With regard to syndrome diagnosis (item #4), we also sent the diagnostic criteria of three major syndromes, ANE [12], AESD [13,14] and MERS [13] (Table 1), together with their typical neuroimaging findings. Diagnosis of hemorrhagic shock and encephalopathy syndrome (HSES) and other syndromes was based on previously published criteria [1,11,15]. As for prognosis (item #6), sequelae were judged as severe if the patient was unable either to walk independently or to utter meaningful words. Responses were sent back either by mail or by fax.

Statistical data were compared among the three syndromes, ANE, AESD and MERS. For numerical data (age), statistical significance was evaluated with one-way ANOVA. The homogeneity of the variances was analyzed by the Levene test; in case of P less than 0.05, pairwise comparisons were made and corrected by Bonferroni method. For categorical data (outcome), we used chi square tests with residual analysis.

This study was based on the Ethical Guideline for Epidemiological Researches published by Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour and Welfare, Japan.

3. Results

3.1. Acute encephalopathy as a whole

Of the 520 hospitals, 265 (51.0%) responded. The total number of cases of acute encephalopathy was 983. The calculated annual incidence was 302 cases per year.

There were 497 males (51.0%) and 477 females (49.0%); no gender difference was noted.

Age at onset ranged from infancy to puberty. The incidence was most high in infancy and early childhood (Fig. 1). The average/standard deviation was 4.0 ± 3.7 years, and the median was 3 years.

Syndrome classification revealed that AESD was the most common (282 cases, 28.7%), followed by MERS (153 cases, 15.6%), ANE (39 cases, 4.0%), HSES (20 cases, 2.0%), limbic encephalitis (15 cases, 1.5%), Reye-like syndrome (7 cases, 0.7%), AERRPS (6 cases, 0.6%), Reye syndrome (4 cases, 0.4%) and posterior reversible encephalopathy syndrome (PRES) (4 cases, 0.4%). Thirteen cases (1.3%) had other syndromes, and 431 cases (43.8%) remained unclassified.

Among pathogenic viruses of preceding infection, influenza virus was the most common (263 cases, 26.6%), followed by HHV-6 (168 cases, 17.0%), rotavirus (40 cases, 4.0%), respiratory syncytial virus (RSV) (17 cases, 1.7%), mumps virus (9 cases, 0.9%), adenovirus (7 cases, 0.7%), HHV-7 (6 cases, 0.6%), HSV (6 cases, 0.6%), norovirus (5 cases, 0.5%), Epstein Barr virus (3 cases, 0.3%), varicella-zoster virus (3 cases, 0.3%), human parechovirus (2 cases, 0.2%) and measles virus (1 case, 0.1%). Bacterial pathogens, such as enterohemorrhagic *Escherichia coli* and *Salmonella*, were detected in 16 cases (1.6%), and *Mycoplasma pneumoniae* in 9 cases (0.9%). Concomitant infections, such as HHV-6/RSV and rotavirus/*Campylobacter jejuni* or *coli*, were found in 5 cases (0.5%). Pathogens remained unidentified in 401 cases (40.8%).

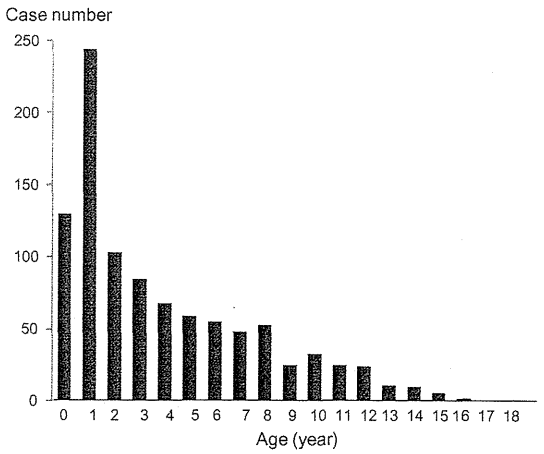

Fig. 1. Age distribution of acute encephalopathy.

The outcome of acute encephalopathy varied. Full recovery was noted in 552 cases (56.2%), mild to moderate sequelae in 218 (22.1%), severe sequelae in 133 (13.5%), and death in 55 (5.6%).

3.2. Major syndromes of acute encephalopathy

3.2.1. AESD

AESD was the most frequent syndrome (282 cases), with 114 male (40.4%) and 167 female (59.6%) patients. Age distribution showed a high incidence in infancy (average/standard deviation 1.7 ± 2.2 years, median 1 year) (Fig. 2).

Pathogens of the preceding infection were HHV-6 in 108 cases (38.2%), influenza virus in 27 (9.5%), HHV-7 in 5 (1.8%), rotavirus in 4 (1.4%) and RSV in 4 (1.4%). There were no cases of bacterial infection.

Outcome of AESD was characterized by low fatality and a high incidence of neurologic sequelae. Full recovery was noted in 81 patients (28.7%), mild to moderate sequelae in 116 (41.1%), severe sequelae in 71 (25.1%) and death in only 4 (1.4%). The ratio of patients with mild to moderate sequelae was significantly higher than for ANE ($P < 0.01$) and MERS ($P < 0.01$).

3.2.2. MERS

MERS was the second most frequent syndrome (153 cases), with 80 male (52.3%) and 69 female (45.1%)

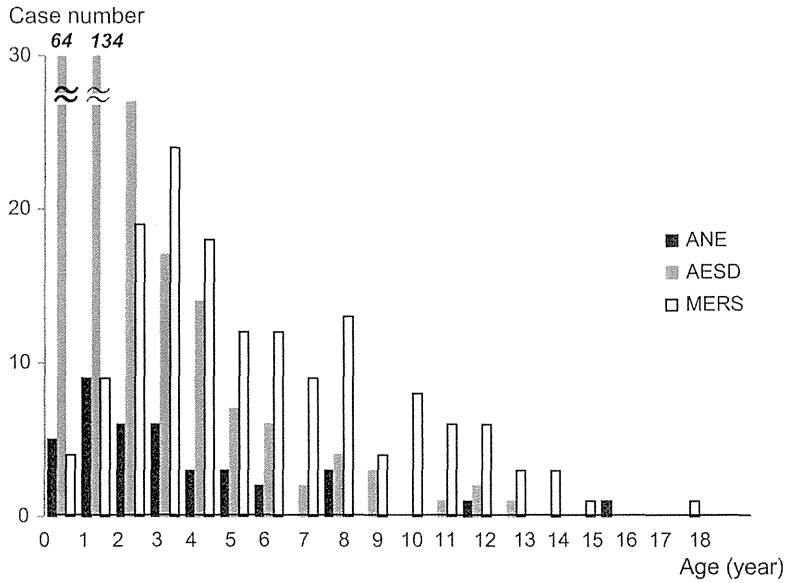

Fig. 2. Age distribution of major syndromes of acute encephalopathy. ANE, acute necrotizing encephalopathy; AESD, acute encephalopathy with biphasic seizures and late reduced diffusion; MERS, mild encephalitis/encephalopathy with a reversible splenial lesion.

patients. Age at onset varied (average/standard deviation 5.6 ± 3.7 years, median 5 years), and was significantly higher than for AESD ($P < 0.01$) (Fig. 2).

Pathogens of the precedent infection were influenza virus in 53 cases (34.4%), rotavirus in 18 (11.7%), mumps virus in 6 (3.9%), and HHV-6 in only 3 (2.0%). Notably, there were 5 cases (3.3%) following bacterial infections.

Outcome was good, with the vast majority of patients (138 cases, 90.2%) achieving a full recovery. The ratio of full recovery was significantly higher in MERS than in AESD ($P < 0.01$). In the remaining patients (11 cases, 7.1%), the sequelae were mild to moderate. There was no case resulting in severe handicap or death.

3.2.3. ANE

ANE ranked third with regard to incidence (39 cases); there were 23 male (59.0%) and 16 female (41.0%) patients. Age at onset of ANE showed the highest incidence in infancy (average/standard deviation 3.3 ± 3.4 years, median 2 years) (Fig. 2), and was significantly higher than for AESD ($P < 0.01$) and lower than for MERS ($P < 0.01$).

Pathogens of the preceding infection were influenza virus in 16 cases (41.0%) and HHV-6 in 8 (20.5%). There was no case of bacterial infection.

Outcome was poor in most patients. Full recovery was noted in only 5 patients (12.8%), mild to moderate sequelae in 9 (23.0%), severe sequelae in 13 (33.3%) and death in 11 (28.2%). Compared to AESD, the mortality of ANE was higher, whereas the probability of neurologic sequelae was comparable. The ratio of full recovery was significantly lower than for AESD ($P < 0.01$) and MERS ($P < 0.01$), and that of death significantly higher than for AESD ($P < 0.01$) and MERS ($P < 0.01$).

3.2.4. HSES

HSES was the fourth most common syndrome (20 cases), with 8 male (40.0%) and 12 female (60.0%) patients. Age at onset ranged from 0 to 8 years. The average and median age was 2.9 ± 2.9 years and 1 year, respectively.

Pathogens of the preceding infection were influenza virus in 3 cases, HHV-6 in 2, norovirus in 1, and RSV in 1.

Outcome was very poor. Eleven patients (55.0%) died, whereas only 2 (10.0%) showed full recovery. The remaining patients had neurologic sequelae, mild to moderate in 1 (5.0%) and severe sequelae in 5 (25.0%).

3.3. Major pathogens of acute encephalopathy

3.3.1. Influenza virus

Influenza virus was the most common pathogen (263 cases), with 153 male (58.2%) and 109 female (41.8%) patients. Age at onset of influenza-associated encephalopathy ranged widely from infancy to puberty (Fig. 2). The mean and median ages were 6.3 ± 3.4 and 6 years, respectively.

Syndrome classification revealed that MERS was the most common (53 cases, 20.2%), followed by AESD (27 cases, 10.3%), ANE (16 cases, 6.1%), HSES (3 cases, 1.1%), Reye, Reye-like and other syndrome (each 1 case, 0.4%). More than half of the patients (158 cases, 60.1%) were unclassified.

The outcome varied. Although many patients achieved a full recovery (199 cases, 75.7%), fatal cases were not uncommon (18 cases, 6.8%). Neurologic sequelae were mild to moderate in 22 patients (8.4%), and severe in 22 (8.4%).

3.3.2. HHV-6

HHV-6 was the second most common pathogen (168 cases), with 73 male (43.5%) and 95 female (56.5%) patients. The vast majority of patients were infants under 2 years of age (Fig. 2). Age at onset of HHV-6-associated encephalopathy (average/standard deviation 0.8 ± 1.1 year, median 1 year) was significantly lower than with influenza-associated encephalopathy ($P < 0.001$).

Among encephalopathy syndromes, AESD was by far the most common (108 cases, 64.3%). Eight patients had ANE (4.8%). Other syndromes, such as MERS (3 cases, 1.8%), HSES (2 cases, 1.2%) and limbic encephalitis (1case, 0.6%), were rare. The number of unclassified cases was smaller (39 cases, 23.2%) than for influenza.

Half of the patients recovered (85 cases, 50.6%). Fatality was low (3 cases, 1.8%); however, many patients were left with neurologic sequelae, being mild to moderate (48 cases, 28.6%) or severe (28 cases, 16.7%).

3.3.3. Rotavirus

Rotavirus was the third most common pathogen (40 cases, 16 male and 23 female). The average and median ages were 2.8 ± 2.4 and 2 years, respectively. Eighteen patients had MERS (45.0%), four AESD (10.0%), and one ANE (2.5%). Full recovery was noted in 28 patients (70.0%), mild to moderate sequelae in 5 (12.5%), severe sequelae in 3 (7.5%), and death in 3 (7.5%).

3.3.4. RSV

RSV was the fourth most common pathogen (17 cases, 4 male and 13 female). The average and median ages were 1.4 ± 0.9 and 1 year, respectively. There were 4 cases of AESD, and 1 case each of MERS and HSES. Full recovery was noted in 12 patients (70.6%), mild to moderate sequelae in 3 (17.6%), severe sequelae in 2 (11.8%), and death in none.

4. Discussion

In this study, the Research Committee on the Etiology, Diagnosis and Treatment of Severe and Intractable Acute Encephalopathy, supported by the Ministry of Health, Labour and Welfare of Japan, conducted a nationwide survey on the epidemiology of acute encephalopathy. In Japan, several studies have previously been performed on the epidemiology of acute encephalitis/encephalopathy [6–10]. All these studies classified encephalitis/encephalopathy pathogenically (virologically), but not syndromically (clinico-pathologically). They paid little attention to the distinction between encephalitis and encephalopathy. Some were performed prior to the advent of clinically useful virological methods, such as immunochromatography (rapid antigen detection) for influenza virus and rotavirus [6,7], resulting in inaccurate virological diagnosis in many cases. The present study is the first to focus on acute encephalopathy, and uses both pathogenic and syndrome classifications.

Our study, however, had several limitations. First, the rate of responding hospitals was not high (51.0%), excluding accurate estimation of the nationwide incidence. Second, this survey was a multi-center study in which many and varied hospitals participated. Among them, the medical activities, including various aspects of diagnosis and treatment, are diverse. Accordingly, the quality of the data obtained in this study are not well guaranteed. For instance, most cases of MERS, as well as many cases of AESD, cannot be properly diagnosed without magnetic resonance imaging (MRI) [13]. Poor access to MRI in some hospitals may cause underdiagnosis of these conditions. In addition, some institutions may have failed to perform proper virological examination for the diagnosis of exanthema subitum. It is thus plausible that several cases of HHV-7-associated encephalopathy were misdiagnosed into HHV-6-associated encephalopathy.

Despite these limitations, this study has several strengths. First, the study area covered all prefectures in Japan. Second, a large number of cases were collected. Third, recent advances in virological examination have facilitated rapid and accurate identification of pathogens. Fourth, diagnostic criteria have recently been established for multiple syndromes [12–14], enabling proper syndrome diagnosis in many cases. Taking advantage of this, this study successfully demonstrated many important features of each syndrome as to its age distribution, relation to pathogens, and prognosis.

Among the three major syndromes, ANE, AESD and MERS, there were striking differences. With regard to age distribution, the mean age was 1.7 years in AESD, 3.3 years in ANE, and 5.6 years in MERS. Most cases of AESD occurred in infancy (0–1 years), and those of ANE in infancy and early childhood (0–5 years). By contrast, MERS was often seen in schoolchildren (Fig. 2). These findings were comparable to those of previous studies on AESD [3,16], ANE [2,12] and MERS [4].

With regard to pathogens of the preceding infection, ANE and MERS were strongly associated with influenza. In AESD, by contrast, HHV-6 was the most common pathogen. The findings of ANE in this study are comparable to those reported in 1990's [2,12]. Comparison with previous data [3,4,16] suggests an increase of influenza-associated MERS and a decrease of influenza-associated AESD in this decade. In this study, it

was noteworthy that five cases of MERS had a preceding bacterial infection. This finding is in agreement with previous data that 6 out of 54 MERS cases were infected with streptococcus and *E. coli* (3 cases each) [13]. In contrast, bacterial pathogens were identified in none of the ANE and AESD cases. Although there have previously been several reports of ANE following bacterial infections [17,18], such cases are exceptional.

The prognosis of ANE and HSES was poor. In many cases, ANE caused either death or neurologic sequelae. The findings were comparable to those in the 1980's and 1990's [2,12], indicating that the overall prognosis of ANE has not been improved substantially despite the efficacy of corticosteroids in some cases [19]. The prognosis of AESD was characterized by low mortality (1.4%) and the high possibility of neurologic sequelae (66.2%). These results are again comparable to those of previous studies [3,16], reflecting the failure of current therapies to protect patients from neurologic damage in AESD. By contrast, the prognosis of MERS was excellent, in agreement with the findings of previous reports [4,13].

A large population (43.1%) of patients remained unclassified into specific syndromes. This group may consist of (1) cases of mild encephalopathy showing no abnormal findings on cranial CT/MRI, (2) cases of unknown or uncommon types of encephalopathy, and (3) cases of MERS, AESD and other syndromes in which proper diagnosis could not be reached.

In this study, we also classified acute encephalopathy based on pathogens [1], and found differences between influenza virus and HHV-6 in age distribution, syndrome, and prognosis. With regard to age, HHV-6-associated encephalopathy was predominantly seen in infants, whereas influenza-associated encephalopathy was prevalent also in older children (Fig. 3). This difference is partially explained on the basis of age predilection of these viruses, namely the incidence of exanthem subitum and influenza in general. As to syndromes, HHV-6 was associated strongly with AESD, but not with MERS. By contrast, influenza was associated with all three major syndromes, AESD, ANE and MERS. Reasons for this discrepancy remain unclear. Multiple factors, such as neurovirulence of these viruses, the host response of inflammatory cytokines, and development of the human brain, may possibly be involved. With regard to prognosis, the number of deaths was higher with influenza-associated encephalopathy, whereas that of neurologic sequelae was higher with HHV-6-associated encephalopathy. These findings may merely reflect the difference in the proportion of syndromes.

In general, the data obtained in this study were comparable to those of previous studies for influenza-associated encephalopathy (1999–2002) [8] and HHV-6 encephalopathy (2003–2004) [10], with regard to the incidence, age distribution and sex ratio. As to the prognosis of influenza-associated encephalopathy, however, mortality has markedly decreased from 30% in 1999–2000 [8] to 7% in 2007–2010. This decline may have resulted from improved treatment and/or the altered incidence of each syndrome.

In conclusion, we conducted a national survey of acute encephalopathy in Japan during three years,

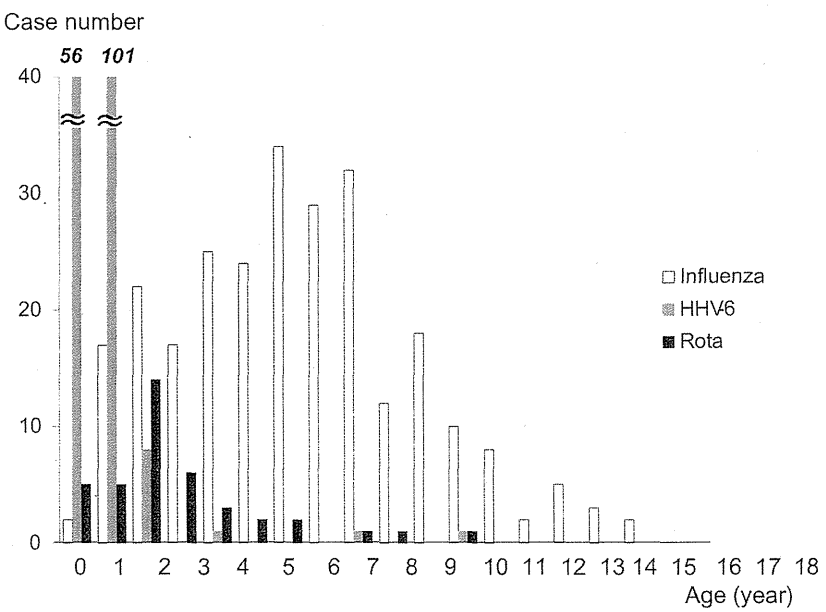

Fig. 3. Age distribution of influenza-, human herpesvirus-6- and Rotavirus-associated encephalopathy HHV-6, human herpesvirus-6; Rota, Rotavirus.

2007–2010, and revealed the epidemiology of ANE, AESD, MERS and other syndromes. These syndromes showed marked differences in their age distribution, pathogens of preceding infection and prognosis, underscoring the necessity for therapies specific to each syndrome.

Acknowledgements

We thank Ms. Kiyomi Noyama and Aya Shoda for their assistance. This study was supported mainly by a Grant-in-aid for Research on Measures for Intractable Diseases, No. H22-Nanji-Ippan-49, from the Ministry of Health, Labour and Welfare, Japan, and partly by Grant-in-Aid for Scientific Research (A) 21249062, (B) 20209753 and (C) 22591176 from the Japan Society for the Promotion of Science, "Research Grants (21B-5) for Nervous and Mental Disorder, Health and Labour Science Research Grant 21210301, KB220001 from the Ministry of Health, Labour and Welfare, Japan, Adaptable and Seamless Technology Transfer Program through Target-driven R&D (A-STEP) Exploratory Research from the Japan Science and Technology Agency, and a research grant from the Japan Epilepsy Research Foundation.

References

[1] Mizuguchi M, Yamanouchi H, Ichiyama T, Shiomi M. Acute encephalopathy associated with influenza and other viral infections. Acta Neurol Scand 2007;186:S45–56.

[2] Mizuguchi M, Abe J, Mikkaichi K, Noma S, Yoshida K, Yamanaka T, et al. Acute necrotising encephalopathy of childhood: a new syndrome presenting with multifocal, symmetric brain lesions. J Neurol Neurosurg Psychiatry 1995;58:555–61.

[3] Takanashi J, Oba H, Barkovich AJ, Tada H, Tanabe Y, Yamanouchi H, et al. Diffusion MRI abnormalities after prolonged febrile seizures with encephalopathy. Neurology 2006;66:1304–9.

[4] Tada H, Takanashi J, Barkovich AJ, Oba H, Maeda M, Tsukahara H, et al. Clinically mild encephalitis/ encephalopathy with a reversible splenial lesion. Neurology 2004;63:1854–8.

[5] Glaser CA, Gilliam S, Schnurr D, Forghani B, Honarmand S, Khetsuriani N, et al. In search of encephalitis etiologies: diagnostic challenges in the California Encephalitis Project, 1998–2000. Clin Infect Dis. 2003;36:731–42.

[6] Ishikawa T, Asano Y, Morishima T, Nagashima M, Sobue G, Watanabe K, et al. Epidemiology of acute childhood encephalitis. Aichi Prefecture, Japan, 1984–90. Brain Dev 1993;15:192–7.

[7] Shiomi M. Acute encephalitis and acute encephalopathy associated with viral infection. In: Education Committee of Japanese Society of Child Neurology, editor. Advances in Child neurology, vol. 29 (in Japanese). Tokyo: Shindan-To-Chiryo-sha; 2000. p. 2–19.

[8] Morishima T. Studies on the epidemiology and pathogenesis of encephalitis/encephalopathy occurring during the clinical course of influenza. In: 2000-2002 General Report of the Research Committee for Research on Emerging and Re-emerging Diseases, (in Japanese). Ministry of Health, Labour and Welfare of Japan, Tokyo: 2003. p. 1–23.

[9] Morishima T. Studies on the pathophysiology, diagnosis and treatment of acute encephalopathy/ encephalitis in children. In: 2005-2007 Report of the Research Supported by Grant-in-Aid for Scientific Research (in Japanese). Japan Society for Promotion of Science, Tokyo: 2009; p. 1–21.

[10] Yoshikawa T, Ohashi M, Miyake F, Fujita A, Usui C, Sugata K, et al. Exanthem subitum-associated encephalitis: nationwide survey in Japan. Pediatr Neurol 2009;41:353–8.

[11] Sakuma H. Acute encephalitis with refractory, repetitive partial seizures. Brain Dev 2009;31:510–4.

[12] Mizuguchi M. Acute necrotizing encephalopathy of childhood: a novel form of acute encephalopathy prevalent in Japan and Taiwan. Brain Dev 1997;19:81–92.

[13] Takanashi J. Two newly proposed infectious encephalitis/encephalopathy syndromes. Brain Dev 2009;31:521–8.

[14] Shiomi S. Therapeutic level theophylline-associated acute encephalopathy with febrile convulsivus status epilepticus. Shonika Rinsho (Tokyo) 2006;59:187–96 (in Japanese).

[15] Chaves-Carballo E, Montes JE, Nelson WB, Chrenka BA. Hemorrhagic shock and encephalopathy. Clinical definition of a catastrophic syndrome in infants. Am J Dis Child 1990;144:1079–82.

[16] Shiomi S. Concept of acute encephalopathy with febrile convulsivus status epilepticus and involvement of theophylline. No To Hattatsu (Tokyo) 2008;40:122–7 (in Japanese).

[17] Uematsu M, Takayanagi M, Nakayama T, Sako M, Yamamoto K, Chikaoka S, et al. A case of acute necrotizing encephalopathy preceded by bacterial meningitis and showing favorable outcome. Nippon Shonika Gakkai Zasshi (Tokyo) 2005;109:735–40 (in Japanese).

[18] Yanagisawa A, Inui T, Namai Y, Takanashi J, Fujii K, Mizuguchi M, et al. Acute necrotizing encephalopathy complicating hemolytic uremic syndrome. Nippon Shoni Jinzobyo Gakkai Zasshi (Tokyo) 2009;22:161–5 (in Japanese).

[19] Okumura A, Mizuguchi M, Kidokoro H, Tanaka M, Abe S, Hosoya M, et al. Outcome of acute necrotizing encephalopathy in relation to treatment with corticosteroids and gammaglobulin. Brain Dev 2009;31:221–7.

Epilepsia, 53(3):558–564, 2012
doi: 10.1111/j.1528-1167.2011.03402.x

FULL-LENGTH ORIGINAL RESEARCH

Mutations of the *SCN1A* gene in acute encephalopathy

*Makiko Saitoh, *Mayu Shinohara, †Hideki Hoshino, †Masaya Kubota, ‡Kaoru Amemiya, §Jun-IchiTakanashi, ¶Su-Kyeong Hwang, ¶Shinichi Hirose, and *Masashi Mizuguchi

*Department of Developmental Medical Sciences, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; †Division of Neurology, National Center for Child Health and Development, Hachioji, Japan; ‡Department of Neurology, Tokyo Metropolitan Hachioji Children's Hospital, Tokyo, Japan; §Department of Pediatrics, Kameda Medical Center, Kamogawa, Japan; and ¶Department of Pediatrics and Research Institute for the Pathomechanisms of Epilepsy, Fukuoka University, Fukuoka, Japan

Summary

Purpose: Acute encephalopathy is the most serious complication of pediatric viral infections, such as influenza and exanthema subitum. It occurs worldwide, but is most prevalent in East Asia. Recently, there have been sporadic case reports of epilepsy/febrile seizure and acute encephalopathy with a neuronal sodium channel alpha 1 subunit (*SCN1A*) mutation. To determine whether *SCN1A* mutations are a predisposing factor of acute encephalopathy, we sought to identify *SCN1A* mutations in a large case series of acute encephalopathy including various syndromes.

Methods: We analyzed the *SCN1A* gene in 87 patients with acute encephalopathy, consisting of 20 with acute necrotizing encephalopathy (ANE), 61 with acute encephalopathy with biphasic seizures and late reduced diffusion (AESD), and six with nonspecific (unclassified) acute encephalopathy.

Key Findings: Three patients had distinct point mutations. Two of them had epileptic seizures prior to acute encephalopathy. Clinical and neuroradiologic findings of acute encephalopathy were diverse among the three patients, although all had a prolonged and generalized seizure at its onset. The first patient with V982L had partial epilepsy and AESD. The second patient with M1977L had febrile seizures and nonspecific acute encephalopathy. The third patient with R1575C had no seizures until the onset of ANE. M1977L was a novel mutation, whereas the remaining two, V982L and R1575C, have previously been reported in cases of Dravet syndrome and acute encephalopathy, respectively.

Significance: These findings provide further evidence that *SCN1A* mutations are a predisposing factor for the onset of various types of acute encephalopathy.

KEY WORDS: *SCN1A*, Ion channel gene defect, Acute encephalopathy status epilepticus, Seizure susceptibility.

Acute encephalopathy (AE) refers to brain dysfunction of acute onset that usually follows an infectious disease with fever. Pathologic substrate of AE is diffuse, noninflammatory brain edema. AE is most common in infants and young children, and is manifested clinically with stupor/coma and a febrile seizure, which is often severe and prolonged.

Based on clinical and neurologic findings, AE is classified into multiple syndromes, such as Reye's syndrome, acute necrotizing encephalopathy (ANE) (Mizuguchi et al., 1995), and acute encephalopathy with biphasic seizures and late reduced diffusion (AESD) (Takanashi et al., 2006). These syndromes show distinct computed tomography/magnetic resonance imaging (CT/MRI) features: diffuse brain edema in Reye's syndrome; bilateral, symmetric thalamic lesions in ANE; and cerebral cortical edema of subacute onset (usually appearing on the third to ninth day of illness, and sparing the perirolandic region) in AESD (Mizuguchi et al., 2007); however, about one-third of patients with AE show no such features and are unable to be classified into these syndromes (nonspecific AE). Pathogenesis of AE is complex, and much remains to be elucidated. The main pathomechanism differs among syndromes: metabolic disorder in Reye's syndrome, cytokine storm in ANE, and excitotoxicity in AESD (Mizuguchi et al., 2007). Delayed neuronal death after a severe/prolonged febrile seizure may play a major role in the pathophysiology of AESD (Mizuguchi et al., 2007; Takanashi et al., 2009).

Mutation of the neuronal sodium channel alpha 1 subunit (*SCN1A*) is the most common cause of hyperthermia-induced seizure susceptibility in patients with generalized epilepsy with febrile seizure plus (GEFS+) and Dravet syndrome (Escayg et al., 2000; Claes et al., 2001; Escayg et al., 2001; Wallace et al., 2001, 2003; Ohmori et al., 2002; Sugawara et al., 2002; Nabbout et al., 2003; Fukuma et al., 2004; Mantegazza et al., 2005; Escayg & Goldin, 2010). By contrast, *SCN1A* mutations are rare in febrile seizures other

Accepted December 20, 2011; Early View publication February 6, 2012.

Address correspondence to Dr. Makiko Saitoh, Department of Developmental Medical Sciences, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo, Tokyo 113-0033, Japan. E-mail: makisaito-tky@umin.net.jp

than GEFS+ and Dravet syndrome (Malacarne et al., 2002). Recently, there have been sporadic case reports of epilepsy/febrile seizure and AE with an *SCN1A* mutation (Sakakibara et al., 2009; Kobayashi et al., 2010; Takayanagi et al., 2010). In addition, children with Dravet syndrome occasionally have AE, which often causes death (Sakauchi et al., 2011). These cases suggest the role of *SCN1A* mutations in the pathogenesis of AE. To validate the hypothesis that *SCN1A* mutations are a predisposing factor of AE, we conducted *SCN1A* gene analysis in a large case series of AE, including various syndromes.

Methods

Patients

The Ethics Committees of the University of Tokyo approved this study. The parents or legal guardians of participants signed an informed consent form as approved by the ethics committees. Eighty-seven patients, 48 female and 39 male, who were diagnosed with AE and treated by 29 hospitals in Japan between September 2008 and August 2010, participated in this study. All the patients were of Japanese ethnicity. Diagnosis of AE was based on the following criteria: (1) impairment of consciousness showing acute onset, rapid progression and duration for more than 24 h, with or without seizures; (2) onset during the course of a febrile and/or infectious disease; and (3) exclusion of similar conditions, such as postictal stupor/coma, effect of anticonvulsants, meningitis, encephalitis, toxic encephalopathy, and metabolic errors. In addition, the patients were divided to three subgroups. Diagnosis of ANE and AESD was based on the criteria described previously (Hoshino et al., 2011). According to syndromic classification, 20 patients had ANE, 61 had AESD, and 6 had nonspecific AE (Table 1). There was no case of classical Reye's syndrome. Seven patients had preexisting febrile seizures and one patient had partial epilepsy before the onset of acute encephalopathy (Table 1). One hundred healthy adult Japanese volunteers without a history of AE served as control subjects.

Genetic analysis

Genomic DNA of patients with AE was prepared from ethylene diamine tetraacetic acid (EDTA)–treated whole blood samples using the QuickGene DNA whole blood kit (Fujifilm Corporation, Tokyo, Japan). *SCN1A* was screened by a direct sequencing method with an automatic sequencer, as described previously (Kobayashi et al., 2010). Reference sequence of mRNA was based on information available from GenBank (accession number: Human SCN1A. AF117907.1).

Results

Of the 87 AE cases studied, three had missense mutations—V982L, M1977L, and R1575C—none of which were found in the 100 controls. The *V982L* mutation was found in case 1 with partial epilepsy and AESD. The valine 982 residue is located on the transmembrane segment 6, domain II of SCN1A protein, is highly conserved among vertebrates, and shares homology with other types of sodium channels (Fig. 1). This mutation was previously reported in a patient with Dravet syndrome without myoclonic seizures and ataxia (Singh et al., 2009). The M1977L mutation was found in case 2 with GEFS+ and nonspecific AE. The methionine 1977 residue is located on near the

Table 1. Clinical data of 87 patients with acute encephalopathy (AE)

Diagnosis	Sex	Age at onset of AE (mean)	Family history of FS/epilepsy	Preexisting seizures	Preceding infection	Status epilepticus or cluster of seizures within 2 days after the onset of AE	Prognosis: Neurologic sequelae
ANE (n = 20)	Male 7 Female 13	8 months to 9 years 7 months (2 years 4 months)	FS 2 Epilepsy 0 None 18	FS 1 Epilepsy 0 None 19	HHV-6 3 Flu 4 RSV 2 Rota 1 NI 10	Yes 3 No 2 NA 15	Severe 9 Mild or none 9 NA 2
AESD (n = 61)	Male 28 Female 33	5 months to 6 years (1 year 9 months)	FS 6 Epilepsy 1 None 50 NA 4	FS 3 Epilepsy 1 None 53 NA 4	HHV-6 18 Flu 8 RSV 2 Others 4 NI 29	Yes 33 No 23 NA 5	Severe 14 Mild or none 41 NA 6
Nonspecific AE (n = 6)	Male 4 Female 2	1 year 9 months to 6 years 1 month (3 years 2 months)	FS 1 None 5	FS 3 None 3	Flu 2 NI 4	Yes 5 No 1	Severe 0 Mild or none 5 NA 1

Patients were classified into three syndromes: acute necrotizing encephalopathy (ANE), acute encephalopathy with biphasic seizures and late reduced diffusion (AESD), and nonspecific AE. FS, febrile seizures; NA, not available; HHV-6, human herpesvirus 6; Flu, influenza virus; RSV, respiratory syncytial virus; Rota, rotavirus; NI, not identified.

	1.V982L	2.M1977L	3.R1575C
SCN1A	VIGNLVVLNLF	KTDLTMSTAAC	TTILSRINLVF
CHIMPANZEE	----------	----------	----------
RAT	--R-------	----------	-S--------
MOUSE	----------	----------	-S--------
DROS	----------		
SCN2A	----------	---M-P--TSP	-N--YW-----
SCN3A	----------	---GSS--TSP	
SCN8A	----------		EN--YW-----

Figure 1.
Alignment of the amino acids surrounding the missense mutations in *SCN1A* of three acute encephalopathy cases. CHIMPANZEE (accession no. XP_515872), *Pan troglodytes* sodium channel α subunit; RAT (NP_110502), rat sodium channel α1 subunit; MOUSE (CAM17350), mouse sodium channel α1 subunit; DROS (NP_523371), *Drosophila melanogaster* sodium channel α subunit. *Drosophila* gene shares no homology with human *SCN1A* (BAC21101) at M1977 and R1575 locations. All other amino acid sequences are from human sodium channel α subunit expressed in the brain. SCN8A (NP_055006) shares no homology with SCN1A at the C-terminal end of the protein. Accession nos. of SCN2A and SCN3A are Q99250 and NP_008853.
Epilepsia © ILAE

C-terminus, and is conserved through mammalians (Fig. 1). The R1575C mutation was found in case 3 with ANE. This mutation has been reported previously in a patient with acute encephalitis with refractory, repetitive partial seizures (AERRPS) (Kobayashi et al., 2010), as well as in a patient with Rasmussen encephalitis with an autoantibody against the glutamate receptor GluR3 (Ohmori et al., 2008), and markedly alters the electrophysiologic properties of the sodium channel. The arginine 1575 residue is highly conserved among vertebrates (Fig. 1). The clinical course of these three patients is described briefly below.

Case 1: AESD with V982L mutation

This patient, a 2-year-old girl, was born small-for-date weighing 2,008 g, after gestation of 39 weeks. There was no family history of epilepsy or seizure disorders. From the age of 3 months, she had recurrent, afebrile complex partial seizures. There was no febrile seizure, myoclonic seizure, mental delay, or ataxia. The diagnosis of partial epilepsy was made, and prophylaxis with carbamazepine was begun. From 1 year and 11 months, she also had bronchial asthma. At 2 years and 3 months of age, she underwent treatment with intravenous theophylline for an asthma attack. She then had a respiratory infection with fever and vomiting, and developed myoclonic seizures, which evolved into prolonged generalized tonic convulsion. Status epilepticus was refractory to anticonvulsants and lasted 2 hours, requiring general anesthesia and mechanical ventilation. Although cranial CT was normal on day 2, coma persisted. Serum levels of aminotransferases and lactic dehydrogenase were very high. On day 5, she had clusters of partial seizures. CT revealed diffuse cerebral cortical edema. Based on the biphasic clinical course and serial CT findings, the diagnosis of AESD was made. She was treated with continuous infusion of thiopental (until day 21) and methylprednisolone pulse therapy. CT and MRI thereafter showed severe atrophy of the cerebral cortex with sparing of bilateral perirolandic regions (Fig. 2). She was eventually left with spastic quadriplegia and severe mental deficit.

Case 2: Nonspecific AE with M1977L mutation

This patient, a 3-year-old boy, was born uneventfully to nonconsanguineous parents. His uncle had had febrile convulsions during infancy. From the age of 1 year 6 months, he had recurrent febrile seizures. At 3 years of age, he had a respiratory infection with fever, and then had his sixth attack consisting of repeated generalized convulsions, six times during 24 h. Each convulsion lasted from several seconds to 5 min. Phenytoin (15 mg/kg) was given intravenously to prevent their recurrence. Consciousness disturbance (Glasgow Coma Scale score, E4V4M5) persisted for 24 h, and was explained neither by postictal stupor nor by sedative effects of phenytoin. Cranial CT revealed mild diffuse edema. Electroencephalography (EEG) on day 1 showed high-voltage slow activity in the right parietal area. He was diagnosed with nonspecific AE and recovered completely; however, he developed a cluster of afebrile seizures 1 month later. EEG showed bilateral frontal spike-waves. Valproate treatment successfully prevented seizures. He had neither mental delay nor ataxia.

Case 3: ANE with R1575C mutation

The patient is now a 12-year-old boy who was born uneventfully and showed normal development. At the age 9 months, he had acute gastroenteritis with fever (tempera-

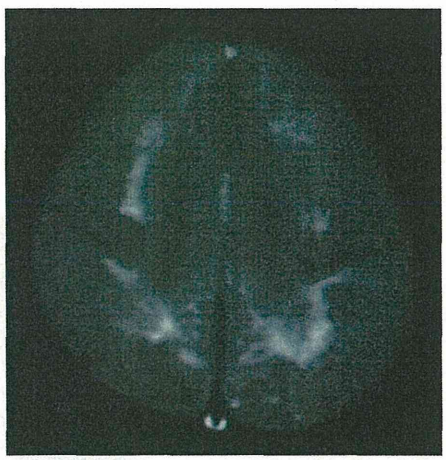

Figure 2.
Cranial MRI of a patient (case 1) with acute encephalopathy with biphasic seiaures and late reduced diffusion (AESD). Imaging at 4 years after AE (fluid-attenuated inversion recovery, FLAIR) showed cerebral atrophy, high signal intensity of the white matter, and sparing of the bilateral perirolandic regions.
Epilepsia © ILAE

ture 38°C) and diarrhea. Four days later, he was taken to hospital because of generalized seizure after several bouts of vomiting. On admission, he was afebrile and mentally obtunded, and had recurrent generalized tonic–clonic seizures. Blood examination showed slight elevation of C-reactive protein, and cerebrospinal fluid examination showed an increased protein level (370 mg/dl). MRI revealed abnormal signals in the bilateral thalamus and subcortical white matter on T_2-weighted images (Fig. 3A). Contrast T_1-weighted imaging showed medullary streaks in the white matter (Fig. 3B). Based on the clinical and imaging findings, the diagnosis of ANE was made. He was treated with intravenous anticonvulsants and gammaglobulin, and recovered without any sequelae. Two years later, his younger sister was also affected by ANE at the age of 8 months, and was eventually left with severe motor and cognitive impairment. She did not have the R1575C mutation.

Discussion

AE is a complex disorder in which multiple factors, both genetic and environmental, are involved. Environmental factors include not only infectious agents, such as influenza virus and human herpesvirus 6 (HHV-6), but also drugs, such as aspirin and theophylline (Mizuguchi et al., 2007). With regard to genetic factors, single nucleotide polymorphisms (SNPs) of carnitine transpalmitoyl transeferase II (*CPTII*) and Toll-like receptor 3 (*TLR-3*) have been identified as predisposing factors of AE (Chen et al., 2005; Hidaka et al., 2006; Shinohara et al., 2011). In our previous study on the *CPT II* gene, we found in two of the present cases (cases 2 and 3) thermolabile SNPs associated with susceptibility to AE (Shinohara et al., 2011). The relationship of these SNPs and AE is complex. For example, *CPTII* SNPs occur in association with two syndromes: AESD and ANE (Shinohara et al., 2011). For each syndrome, unidentified genes other than *CPTII* are likely to be also involved. On the other hand, there is one syndrome of AE caused by mutations of a single gene: *ANE1* (familial recurrent variant of ANE) due to mutation of the Ran-binding protein 2 (*RANBP2*) gene (Neilson et al., 2009).

In this study, we tested the possibility that mutation of another gene, *SCN1A*, is a genetic predisposition for the onset of AE. We performed *SCN1A* gene analysis in 87 Japanese patients with AE, and found point mutations, V982L, M1977L, and R1575C, in three (3.4%) of them. These results are comparable to those of a previous study, which found an *SCN1A* mutation in one of 15 AE patients (Kobayashi et al., 2010). Our study dealt with a large case series of AE, and found multiple patients with *SCN1A* mutations, thereby establishing the association between *SCN1A* mutations and AE.

In addition to our three patients, three additional patients with AE and a *SCN1A* mutation have been reported previously. Clinical and neuroradiologic findings of these six patients are variable (Table 2). With regard to epilepsy, two patients had Dravet syndrome, one had GEFS+, one had partial epilepsy, one had febrile seizures, and the remaining one had none. On the other hand, *SCN1A* missense mutations have been identified not only in patients with Dravet syndrome and GEFS+, but also in unaffected subjects without a seizure disorder (Ohmori et al., 2008). Therefore, their contribution to epilepsy and AE requires critical evaluation and further investigation.

With regard to AE, three had AESD, one had AERRPS, one had ANE, and one had nonspecific AE. Clinical features varied among the cases, although all had either prolonged seizures (status epilepticus; four cases) or a cluster of brief seizures (three cases), mostly with fever (five cases), on day 1 or 2, in contrast to mutation-negative patients in whom such seizures were absent in about 40% (Table 1). Prognosis also varied largely from severe psychomotor deficit to complete recovery. Notably, two patients with the same *SCN1A* mutation, R1575C, showed clinical phenotypes quite different from each other. Case 3 in this study had no

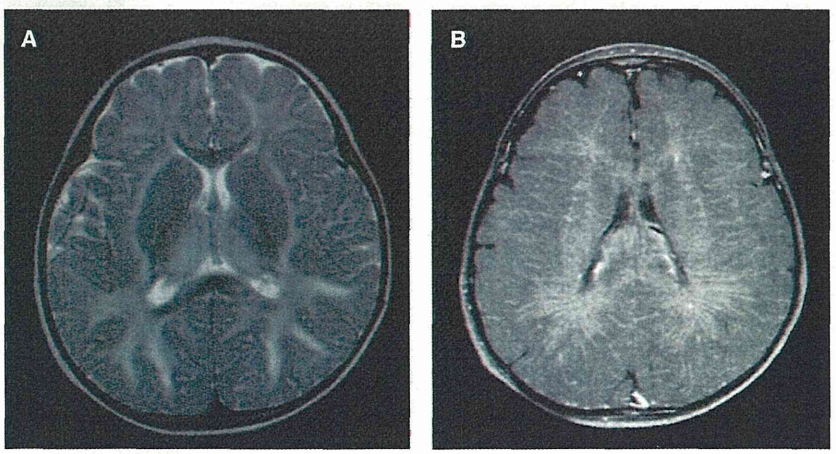

Figure 3.
Cranial MRI of a patient (case 3) with acute necrotizing encephalopathy (ANE). (**A**) T_2-weighted axial section shows high-intensity areas of bilateral thalami and the cerebral white matter. (**B**) Gadolinium-enhanced T_1-weighted axial image shows medullary streaks in the affected white matter lesions.