Journal of Clinical Microbiology (2004. Aug) 42(8):3891-3893.

Solitary neurocysticercosis case caused by Asian genotype of Taenia solium confirmed by mitochondrial DNA analysis.

Yamasaki, Hiroshi ; Matsunaga, Shigeo ; Yamamura, Koji ; Chang, Chia-Cheng ; Kawamura, Shunji ; Sako, Yasuhito ; Nakao, Minoru ; Nakaya, Kazuhiro ; Ito, Akira

JOURNAL OF CLINICAL MICROBIOLOGY, Aug. 2004, p. 3891–3893 Vol. 42, No. 8
0095-1137/04/$08.00+0 DOI: 10.1128/JCM.42.8.3891–3893.2004

Solitary Neurocysticercosis Case Caused by Asian Genotype of *Taenia solium* Confirmed by Mitochondrial DNA Analysis

Hiroshi Yamasaki,[1]* Shigeo Matsunaga,[2]† Koji Yamamura,[2] Chia-Cheng Chang,[2] Shunji Kawamura,[3]‡ Yasuhito Sako,[1] Minoru Nakao,[1] Kazuhiro Nakaya,[4] and Akira Ito[1]

Department of Parasitology[1] and Animal Laboratory for Medical Research,[4] Asahikawa Medical College, Asahikawa, and Department of Neurosurgery, Yokohama Minami Kyosai Hospital,[2] and Department of Pathology, Yokohama Rosai Hospital,[3] Yokohama, Japan

Received 25 December 2003/Returned for modification 31 March 2004/Accepted 20 April 2004

A Japanese woman presenting with neurologic symptoms was presumptively diagnosed with neurocysticercosis based on imaging findings. Hooklets in the scolex of the resected lesion were not confirmed through histopathological observation. However, the illness was confirmed by mitochondrial DNA analysis to be a solitary neurocysticercosis case caused by the Asian genotype of *Taenia solium*.

CASE REPORT

A 53-year-old Japanese woman developed aphasia and numbness of the right arm beginning 29 November 2002. The patient was admitted to the Department of Neurosurgery, Yokohama Minami Kyosai Hospital, Yokohama, Japan, on 16 December 2002 with complaints of aphasia and numbness of the right arm. On admission, cerebral computed tomography (CT) showed a low-density area in the left frontal lobe (Fig. 1A), and the lesion appeared as a ringlike small mass (hole-with-dot imaging) with a diameter of 2 cm surrounded with edema at the same site in the CT scan with contrast enhancement (Fig. 1B). Magnetic resonance imaging (MRI) showed the lesion with a low-intensity signal in a T1-weighted image (Fig. 1C) and a high-intensity signal in a T2-weighted image (Fig. 1D). Perifocal edema is evident in the T2-weighted image. The lesion was enhanced after administration of gadolinium-diethylenetriaminepentaacetic acid (Fig. 1E and F). Neurocysticercosis (NCC) caused by a *Taenia solium* cysticercus was strongly suspected based on the imaging findings, and total removal of the mass was performed for differentiation from a tumor (Fig. 2A). The mass was located near the surface of the brain and had prominent surrounding gliosis. Histopathological examination of the resected lesion revealed suckers and a spiral canal unique to the taeniid cysticercus, but no hooklet was observed in any section (Fig. 2B). Postoperative examinations for screening of other visceral organs including X rays of the extremities and the whole body showed no other abnormality. After surgery, the patient recovered well with no residual deficit, resuming her preillness activity. She repeated extensive travels to France, Spain, Portugal, India, and Southeast Asian countries (Thailand, Myanmar, Vietnam, and Malaysia) that she had taken during the period from 1993 to 2001.

Serological examination and mitochondrial DNA analysis. A blood sample was obtained from the patient with informed consent according to guidelines from institutional review boards at Yokohama Minami Kyosai Hospital. Serological confirmation of immunoblots using both purified glycoproteins (5) and a recombinant chimeric antigen (9) was carried out at Asahikawa Medical College before surgical operation; however, there was no detectable specific antibody response against either antigen (data not shown). For definitive diagnosis of the causative agent, mitochondrial DNA analysis was performed using a small piece of a formalin-fixed, paraffin-embedded specimen. The paraffin was melted in a heat block at 70°C, and a tiny amount of parasite material was separated. The parasite was lysed in 60 μl of 0.02 N sodium hydroxide containing proteinase K at 90°C for 15 min. After removal of the proteinase K by use of phenol-chloroform, the resulting solution was used directly as template DNA for the amplification of the cytochrome *c* oxidase subunit 1 gene (*cox1*). Two products, one of approximately 1.6 kb and one of 984 bp, were successfully amplified by using 5′-TTGTTATAAATTTTTGATTACTAAC-3′ (16) as the forward primer and 5′-TCCACTAAGCATAATGCAAAAGGC-3′ (7) and 5′-GACATAACATAATGAAAATG-3′, respectively, as the reverse primers (reference 16 and data not shown). The PCR protocol consisted of 35 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 2 min plus 1 cycle of 72°C for 5 min using the Ex *Taq* DNA polymerase Hot Start version (Takara Bio Inc., Shiga, Japan). The samples for DNA sequencing were prepared using the ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction kit, and DNA sequencing was performed on an ABI PRISM 310 Genetic Analyzer.

In Fig. 3, the partial nucleotide sequence of the taeniid specimen is aligned with nucleotide sequences of *cox1* from

* Corresponding author. Mailing address: Department of Parasitology, Asahikawa Medical College, Midorigaoka Higashi 2-1-1-1, Asahikawa 078-8510, Japan. Phone: 81 166 68 2421. Fax: 81 166 68 2429. E-mail: hyamasak@asahikawa-med.ac.jp.

† Present address: Department of Neurosurgery, National Hospital, Yokohama Medical Center, Yokohama 245-8575, Japan.

‡ Present address: Department of Pathology, Tokyo Women's Medical University, Tokyo 162-8666, Japan.

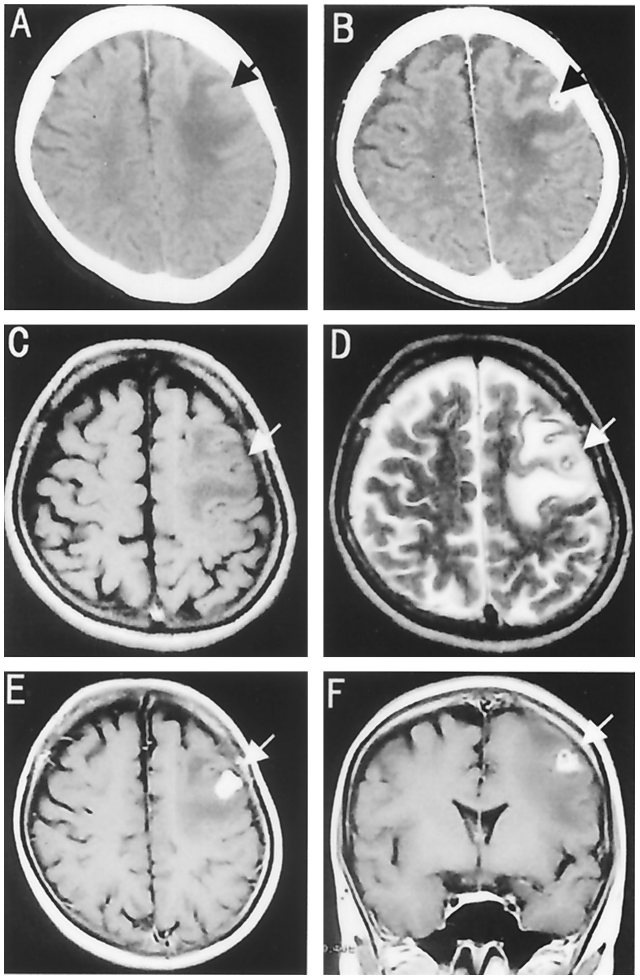

FIG. 1. Neuroimaging findings on admission of patient. Plain CT scan showing a low-density area in the left frontal lobe (A) and well-defined lesion in enhanced CT scan with contrast enhancement (B). MRI showing the lesion with low-intensity signal on T1-weighted image (C) and high-intensity signal on T2-weighted image (D). The lesion is enhanced with gadolinium-diethylenetriaminepentaacetic acid in the axial (E) and coronal (F) views.

human taeniid cestodes. Although some differential nucleotides are dispersed over the *cox1* sequences, the representative three nucleotides at positions 672, 690, and 723 are shown as diagnostic markers for taeniid species or *T. solium* genotypes. A nucleotide at position 672 is for differentiation of taeniid species, and the other two, at positions 690 and 723, are pertinent for differentiation of two genotypes of *T. solium* (7, 15). In this case, the nucleotides at positions 690 and 723 are guanine and cytosine, respectively, indicating that the resected cysticercus has the Asian genotype of *T. solium*. Consideration of the patient's travel history revealed possibilities of exposure to *T. solium* eggs during her stays in India or other Southeast Asian countries where *T. solium* NCC is still endemic.

NCC is one of the most serious parasitic diseases of public health importance and is currently recognized as a reemerging disease in both developed and developing countries (1, 10, 11, 12). Imaging diagnosis of NCC using CT and MRI is routinely performed, and approximately 10% of NCC cases are easily diagnosed based upon imaging findings (2). Serological examination is also reliable and sensitive for NCC patients with multiple cysts but not for those with a solitary cyst (3, 8, 13, 14). As in this case, it is not always possible to observe the characteristic hooklets of *T. solium* through histopathological examination due to either technical problems or incomplete formation of hooklets (6). Moreover, there might be anticipated cysticercosis caused by taeniid species of zoonotic origins other than *T. solium*. We are also interested in molecular evaluation of the racemose form of cysticerci, *Cysticercus racemosus*. Thus, mitochondrial DNA analysis is useful for routine identification of taeniid

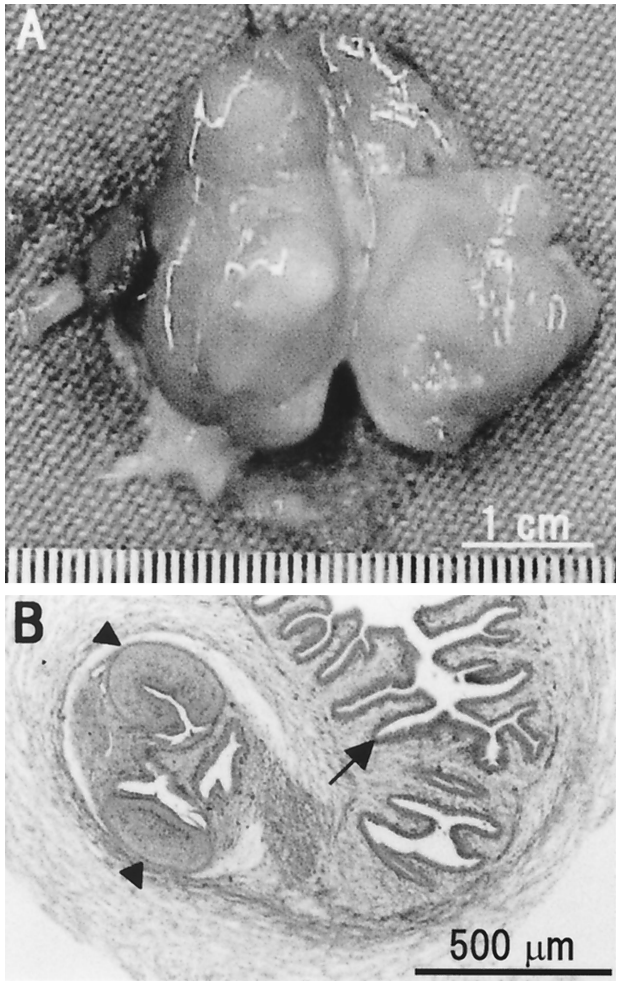

FIG. 2. Macroscopic appearance and histopathological examination section of the resected lesion. (A) The nodule was encapsulated with a delicate membrane. (B) In a section stained with hematoxylin and eosin, the invaginated scolex with suckers (arrowheads) and spiral canal (arrow) are shown. No hooklet was observed. Original magnification, ×25.

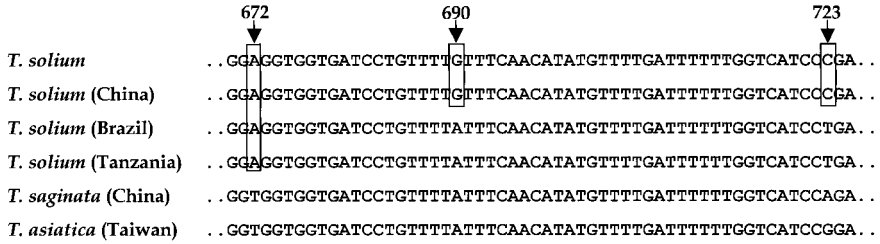

FIG. 3. Alignment of the partial nucleotide sequences of *cox1* from human taeniid cestodes. *T. solium*-specific and *T. solium* genotype-specific nucleotides are boxed and marked with arrows. The numbers indicate the nucleotide positions in the 1,620-bp *cox1* gene. The nucleotide sequence of the causative agent in the case described in the present paper is shown on the top. The nucleotide sequences from the Asian genotype of *T. solium* (from China), the American-African genotypes of *T. solium* (from Brazil and Tanzania), *Taenia saginata* (from China), and *Taenia asiatica* (from Taiwan) are from the DDBJ database with accession numbers of AB066485, AB066492, AB066493, AB066495, and AB066494, respectively.

cestodes and particularly for correlation between pathogenicity and genotypes of *T. solium* (4) when parasite materials are available.

This work was supported in part by a grant-in-aid for scientific research from the Japan Society for Promotion of Science (grant no. 14256001) to A.I.

REFERENCES

1. **Craig, P. S., M. T. Rogan, and J. C. Allan.** 1996. Detection, screening and community epidemiology of taeniid cestode zoonoses: cystic echinococcosis, alveolar echinococcosis and neurocysticercosis. Adv. Parasitol. **38:**169–250.
2. **Del Brutto, O. H., J. Sotelo, and G. C. Román.** 1998. Neurocysticercosis: a clinical handbook. Swets & Zeitlinger, Lisse, The Netherlands.
3. **Ito, A., M. Nakao, Y. Ito, I. Yuzawa, H. Morishima, N. Kawano, and K. Fujii.** 1999. Neurocysticercosis case with a single cyst in the brain showing dramatic drop in specific antibody titers within 1 year after curative surgical resection. Parasitol. Int. **48:**95–99.
4. **Ito, A., M. Nakao, M. Okamoto, Y. Sako, and H. Yamasaki.** 2002. Mitochondrial DNA of *Taenia solium*: from basic to applied science, p. 25–31. *In* G. Singh and S. Prabhakar (ed.), *Taenia solium* cysticercosis: from basic to clinical science. CABI Press, Oxon, United Kingdom.
5. **Ito, A., A. Plancarte, L. Ma, Y. Kong, A. Flisser, S. Y. Cho, Y. H. Liu, S. Kamhawi, M. W. Lightowlers, and P. M. Schantz.** 1998. Novel antigens for neurocysticercosis: simple method for preparation and evaluation for serodiagnosis. Am. J. Trop. Med. Hyg. **59:**291–294.
6. **Margono, S. S., A. Ito, M. O. Sato, M. Okamoto, R. Subahar, H. Yamasaki, A. Hamid, T. Wandra, W. H. Purba, K. Nakaya, M. Ito, P. S. Craig, and T. Suroso.** 2003. *Taenia solium* taeniasis/cysticercosis in Papua, Indonesia in 2001: detection of human worm carriers. J. Helminthol. **77:**39–42.
7. **Nakao, M., M. Okamoto, Y. Sako, H. Yamasaki, K. Nakaya, and A. Ito.** 2002. A phylogenetic hypothesis for the distribution of two genotypes of the pig tapeworm *Taenia solium* worldwide. Parasitology **124:**657–662.
8. **Ohsaki, Y., A. Matsumoto, K. Miyamoto, N. Kondoh, T. Araki, A. Ito, and K. Kikuchi.** 1999. Neurocysticercosis without detectable specific antibody. Intern. Med. **38:**67–70.
9. **Sako, Y., M. Nakao, T. Ikejima, X. Z. Piao, K. Nakaya, and A. Ito.** 2000. Molecular characterization and diagnostic value of *Taenia solium* low-molecular-weight antigen genes. J. Clin. Microbiol. **38:**4439–4444.
10. **Schantz, P. M., A. C. Moore, J. L. Munoz, B. J. Hartman, J. A. Schaefer, A. M. Aron, D. Persaud, E. Sarti, M. Wilson, and A. Flisser.** 1992. Neurocysticercosis in an Orthodox Jewish community in New York City. N. Engl. J. Med. **327:**692–695.
11. **Schantz, P. M., P. P. Wilkins, and V. C. W. Tsang.** 1998. Immigrants, imaging, and immunoblots: the emergence of neurocysticercosis as a significant public health problem, p. 213–242. *In* W. M. Scheld, W. A. Craig, and J. M. Hughes (ed.), Emerging infections 2. ASM Press, Washington, D.C.
12. **Simanjuntak, G. M., S. S. Margono, M. Okamoto, and A. Ito.** 1997. Taeniasis/cysticercosis in Indonesia as an emerging disease. Parasitol. Today **13:** 321–323.
13. **Tsang, V. C., J. A. Brand, and A. E. Boyer.** 1989. An enzyme-linked immunoelectrotransfer blot assay and glycoprotein antigens for diagnosing human cysticercosis (*Taenia solium*). J. Infect. Dis. **159:**50–59.
14. **Wilson, M., R. T. Bryan, J. A. Fried, D. A. Ware, P. M. Schantz, J. B. Pilcher, and V. C. Tsang.** 1991. Clinical evaluation of the cysticercosis enzyme-linked immunoelectrotransfer blot in patients with neurocysticercosis. J. Infect. Dis. **164:**1007–1009.
15. **Yamasaki, H., M. Nakao, Y. Sako, K. Nakaya, M. O. Sato, W. Mamuti, M. Okamoto, and A. Ito.** 2002. DNA differential diagnosis of human taeniid cestodes by base excision sequence scanning thymine-base reader analysis with mitochondrial genes. J. Clin. Microbiol. **40:**3818–3821.
16. **Yamasaki, H., J. C. Allan, M. O. Sato, M. Nakao, Y. Sako, K. Nakaya, D.-C. Qiu, W. Mamuti, P. S. Craig, and A. Ito.** 2004. DNA differential diagnosis of taeniasis and cysticercosis by multiplex PCR. J. Clin. Microbiol. **42:**548–553.