

First Contemporary Case of Human Infection with *Cryptococcus gattii* in Puget Sound: Evidence for Spread of the Vancouver Island Outbreak[∇]

Arlo Upton,¹ James A. Fraser,^{2,3} Sarah E. Kidd,⁴ Camille Bretz,¹ Karen H. Bartlett,⁴
 Joseph Heitman,³ and Kieren A. Marr^{1,5*}

Clinical Research Division, Fred Hutchinson Cancer Research Center and University of Washington, Seattle, Washington¹; University of Queensland, Molecular and Microbial Sciences, Brisbane, Australia²; Department of Molecular Genetics and Microbiology, Duke University Medical Center, Durham, North Carolina³; School of Occupational and Environmental Hygiene, University of British Columbia, Vancouver, BC V6T 1Z3, Canada⁴; and Department of Medicine, Oregon Health and Science University, Portland, Oregon⁵

Received 17 March 2007/Returned for modification 2 June 2007/Accepted 13 June 2007

We report a case of cryptococcosis due to *C. gattii* which appears to have been acquired in the Puget Sound region, Washington State. Genotyping confirmed identity to the predominant Vancouver Island genotype. This is the first documented case of human disease by the major Vancouver Island emergence strain acquired within the United States.

In 2001, an emergence of *Cryptococcus gattii* on Vancouver Island, British Columbia, Canada, was identified, with clinical cases in both humans and other animals dating back to 1999 (2, 3, 7, 12). Environmental sampling identified *C. gattii* associated with trees and with the soil, debris, and air around the trees. Genotypic analysis revealed that virtually all environmental and clinical isolates belong to the VGII genotype of *C. gattii* (7). Studies using PCR fingerprinting, restriction fragment length polymorphism (RFLP) analysis, and amplified fragment length polymorphism (AFLP) analysis (7) identified two distinct genotypes among the isolates from Vancouver Island (VGIIa/AFLP6a and VGIIb/AFLP6B), which were later supported by multilocus sequence typing (MLST) (4, 6). Given the close proximity, climatic and geographic similarities to Vancouver Island, and potential clinical importance, we have sought to determine whether this pathogenic microbe has spread to the Puget Sound area in the United States.

Firstly, in order to determine whether *C. gattii* had been present in the Puget Sound region in Washington, we performed a retrospective chart review of cases of cryptococcosis from 1 January 1997 to 31 December 2004 at two large tertiary Seattle hospitals, Harborview Medical Center and the University of Washington Medical Center. Patients were identified by using discharge codes and were excluded if they had not lived in the Puget Sound area in the 6 months before presentation with cryptococcosis. Our second approach was to perform prospective surveillance of cases of cryptococcosis at Harborview Medical Center and University of Washington Medical Center, starting in January 2005. Lastly, we performed environmental sampling of trees, debris, and soil in the Puget Sound area, using published methodology (7, 11). These studies were ap-

proved by the Fred Hutchinson Cancer Research Center institutional review board.

The retrospective chart review identified 49 new cases of cryptococcosis; none were due to *C. gattii*. Prospective surveillance from January 2005 identified one case of cryptococcosis due to *C. gattii* in a 74-year-old man with a 5-year history of large granular lymphocytic leukemia for which he was being treated with oral prednisone, 2.5 mg daily, and intermittent erythropoietin and pegfilgrastim. He presented in February 2006 with a cough. Radiology revealed a 13-mm lingular nodule, and a thoroscopic wedge resection was performed. The histopathologic findings were consistent with an infectious process, and *C. gattii* was isolated. RFLP and MLST analyses confirmed that the isolate belongs to molecular type VGIIa, the predominant *C. gattii* genotype in the Vancouver Island emergence, and shares 100% nucleotide identity at 28 MLST markers with the Vancouver Island major emergence clinical reference isolate (A1MR265) (Fig. 1). Serum and cerebrospinal fluid cryptococcal antigen titers were negative.

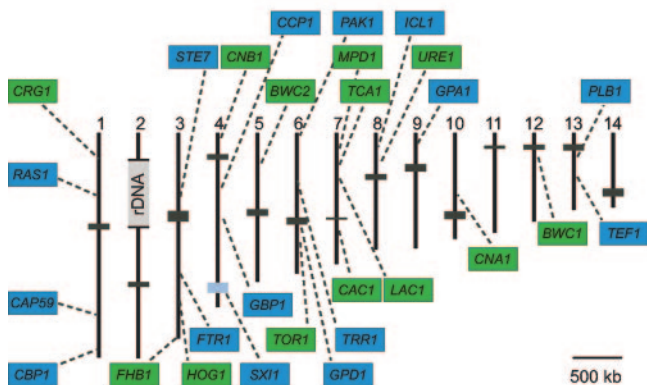
The patient lives on Orcas Island in the Puget Sound with his wife. In the past year they had traveled to Southern California and Hawaii. Their home is in a semirural setting with numerous Douglas fir trees on the property. To determine whether *C. gattii* has established itself in this locale, environmental sampling was undertaken at his property. Sampling in April 2006 of Douglas fir trees ($n = 366$) and underlying soil/debris ($n = 366$) and of gardening soil ($n = 4$) did not yield *C. gattii*.

We present a case of proven human cryptococcosis caused by *C. gattii*, with confirmation of infection with the VGIIa Vancouver Island emergence major genotype. While the patient had visited Los Angeles in the year before presentation, and a single isolate of *C. gattii* identical to the VGIIa Vancouver Island emergence major genotype was identified from an environmental isolate in 1992 from the San Francisco area (4, 6, 10), we believe that acquisition on or near Orcas Island provides a more parsimonious explanation than acquisition in California. The incubation period from exposure to illness is known to average 4 to 6 months, consistent with recent expo-

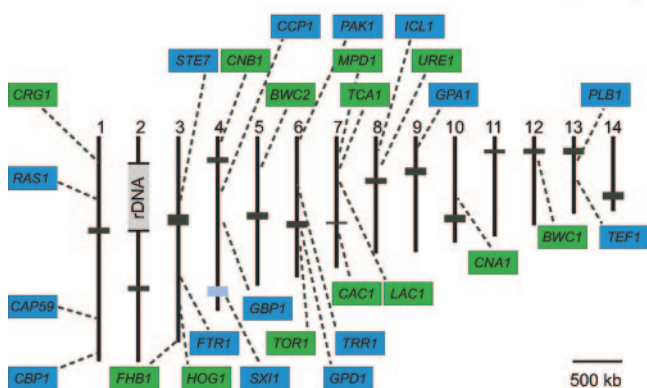
* Corresponding author. Mailing address: Oregon Health and Science University, Division of Infectious Diseases, 3181 SW Sam Jackson Park Rd., Portland, OR 97239. Phone: (503) 418-1685. Fax: (503) 494-4264. E-mail: marrki@ohsu.edu.

[∇] Published ahead of print on 27 June 2007.

Orcas Island T67707 VGIIa genotype isolate



VGIIa R265 - Common on Vancouver Island (95%)



VGIIb R272 - Rare on Vancouver Island (5%) Common in Australia

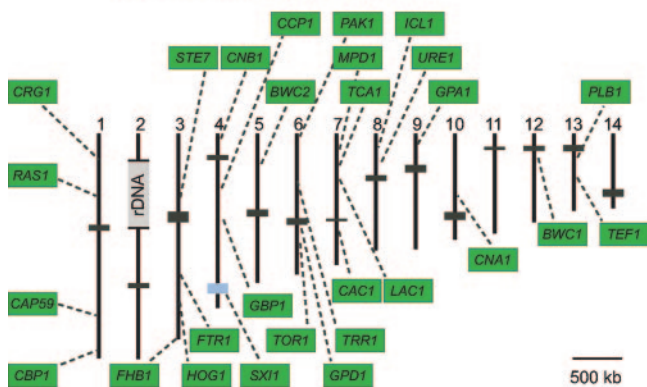


FIG. 1. The Orcas Island strain is a VGIIa genotype isolate: MLST analysis of 28 loci revealed that isolate T67707 is identical in these genomic locations to the VGIIa isolates of Vancouver Island. The locations of each sequenced locus in the related *C. neoformans* JEC21 genome are shown, scattered over 11 of the 14 chromosomes. Loci in green are alleles common to the VGIIb genotype, found on VI and in Australia. Blue loci are unique to the VI VGIIa genotype.

sure, and he had not traveled to Vancouver Island or elsewhere outside the United States. We have been unable to isolate *C. gattii* from the environment around his home; therefore, the source of infection remains unclear. *C. gattii* may have been transiently present in the area at the time of disease acquisition or may be present below the limits of detection, which is consistent with theories of recent dispersal (5, 8).

The expansion of the *C. gattii* niche beyond Vancouver Island is further supported by recent accounts of human cases of *C. gattii* infection without travel to Vancouver Island identified on mainland British Columbia (9). In addition, *C. gattii* (genotyping pending) infection has been diagnosed in four porpoises found washed up on Washington State beaches since 2004 (personal communication from Stephen Raverty, BC Department of Veterinary Health). *C. gattii* infection has also been diagnosed in three domestic cats from Whatcom County, Washington State (9); isolates were determined to be the VGIIa genotype based on analysis of DNA from formalin-fixed, paraffin-embedded tissue. Insufficient material was recovered for MLST analysis, and thus, it is unknown whether these isolates are molecularly identical to isolates from the Vancouver Island emergence. Interestingly, *C. gattii* VGIIa (identical to the Vancouver Island emergence major genotype) isolates have been identified from soil and a fence post swab in northern Washington in 2005 (9).

Two recent cases of *C. gattii* infection have been reported in patients from Oregon State. Both were caused by VGII strains, but based on six-marker MLST analysis, both are different from the major (VGIIa) and minor Vancouver Island (VGIIb) emergence genotypes (9). Thus, the relationship of these *C. gattii* cases in Oregon to the Vancouver Island emergence is unclear. In contrast, our patient's isolate (T67707) belongs to the VGIIa genotype, confirmed using RFLP and 28-marker MLST. Thus, our patient is the first contemporary case of human infection caused by the Vancouver Island emergence strain within the United States. We note that a clinical isolate (NIH444) obtained from a patient in Seattle in the 1970s is also identical, based on MLST analysis, to the Vancouver Island major emergence isolate, but no travel history is available for this historical case (1, 4).

The Vancouver Island emergence is associated with the highest reported incidence of human cryptococcosis in the world, with significant morbidity and mortality (9). Continued surveillance of human and veterinary cases and environmental sampling in the Pacific Northwest are essential to provide more understanding of this emerging infection.

We acknowledge Stephen Raverty for information regarding aquatic mammals and the University of Washington Medical Center Clinical Microbiology Laboratory, where the patient's *C. gattii* was isolated and identified. The authors also acknowledge Angela Talley for her assistance with surveillance and management of clinical cases. In addition, we thank Jim Kronstad for general laboratory support of S.E.K.

The work was supported by NIH/NIAID R01 grant AI39115 to J.H. We have no conflicts of interest.

REFERENCES

1. Boekhout, T., B. Theelen, M. Diaz, J. W. Fell, W. C. Hop, E. C. Abeln, F. Dromer, and W. Meyer. 2001. Hybrid genotypes in the pathogenic yeast *Cryptococcus neoformans*. *Microbiology* 147:891-907.
2. Duncan, C., H. Schwantje, C. Stephen, J. Campbell, and K. Bartlett. 2006. *Cryptococcus gattii* in wildlife of Vancouver Island, British Columbia, Canada. *J. Wildl. Dis.* 42:175-178.

3. **Duncan, C. G., C. Stephen, and J. Campbell.** 2006. Evaluation of risk factors for *Cryptococcus gattii* infection in dogs and cats. *J. Am. Vet. Med. Assoc.* **228**:377–382.
4. **Fraser, J. A., S. S. Giles, E. C. Wenink, S. G. Geunes-Boyer, J. R. Wright, S. Diezmann, A. Allen, J. E. Stajich, F. S. Dietrich, J. R. Perfect, and J. Heitman.** 2005. Same-sex mating and the origin of the Vancouver Island *Cryptococcus gattii* outbreak. *Nature* **437**:1360–1364.
5. **Kidd, S. E., Y. Chow, S. Mak, P. J. Bach, H. Chen, A. O. Hingston, J. W. Kronstad, and K. H. Bartlett.** 2007. Characterization of environmental sources of the human and animal pathogen *Cryptococcus gattii* in British Columbia, Canada, and the Pacific Northwest of the United States. *Appl. Environ. Microbiol.* **73**:1433–1443.
6. **Kidd, S. E., H. Guo, K. H. Bartlett, J. Xu, and J. W. Kronstad.** 2005. Comparative gene genealogies indicate that two clonal lineages of *Cryptococcus gattii* in British Columbia resemble strains from other geographical areas. *Eukaryot. Cell* **4**:1629–1638.
7. **Kidd, S. E., F. Hagen, R. L. Tschärke, M. Huynh, K. H. Bartlett, M. Fyfe, L. Macdougall, T. Boekhout, K. J. Kwon-Chung, and W. Meyer.** 2004. A rare genotype of *Cryptococcus gattii* caused the cryptococcosis outbreak on Vancouver Island (British Columbia, Canada). *Proc. Natl. Acad. Sci. USA* **101**:17258–17263.
8. **Kidd, S. E., J. B. Paxton, A. O. Hingston, S. Mak, Y. Chow, L. Macdougall, J. W. Kronstad, and K. Bartlett.** 2007. *Cryptococcus gattii* dispersal mechanisms, British Columbia, Canada. *Emerg. Infect. Dis.* **13**:51–57.
9. **MacDougall, L., S. E. Kidd, E. Galanis, S. Mak, M. J. Leslie, P. R. Cieslak, J. W. Kronstad, M. G. Morshed, and K. H. Bartlett.** 2007. Spread of *Cryptococcus gattii* in British Columbia, Canada, and detection in the Pacific Northwest, USA. *Emerg. Infect. Dis.* **13**:42–50.
10. **Pfeiffer, T., and D. Ellis.** 1991. Environmental isolation of *Cryptococcus neoformans gattii* from California. *J. Infect. Dis.* **163**:929–930.
11. **Staib, F., M. Seibold, E. Antweiler, B. Fröhlich, S. Weber, and A. Blisse.** 1987. The brown colour effect (BCE) of *Cryptococcus neoformans* in the diagnosis, control and epidemiology of *C. neoformans* infections in AIDS patients. *Zentralbl. Bakteriolog. Mikrobiol. Hyg. A* **266**:167–177.
12. **Stephen, C., S. Lester, W. Black, M. Fyfe, and S. Raverty.** 2002. Multispecies outbreak of cryptococcosis on southern Vancouver Island, British Columbia. *Can. Vet. J.* **43**:792–794.