Cryptococcus gattii outbreak expands into the Northwestern United States with fatal consequences

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Abstract

In the past decade, the primary fungal pathogen Cryptococcus gattii has evolved and adapted to the temperate climate of the Pacific Northwest region of North America. This pathogen is now endemic and an increasingly common cause of life-threatening pulmonary and central nervous system infections that are difficult to manage and, in some cases, fatal to humans and other mammals throughout the region. A series of recent reports provide evidence that evolutionary, climatic, and anthropogenic factors may be causing the expansion of the Vancouver Island outbreak genotype into the United States, with the concomitant emergence of a unique genotype in the state of Oregon. Ongoing studies address the molecular epidemiology, roles of mating and genetic exchange, and geographic origins of this unprecedented outbreak of fungal infection of considerable public health magnitude.

Introduction and context

During the past decade (1999 to 2009) Cryptococcus gattii has emerged as a primary pathogen in western North America, including both Canada and the United States [1-4]. This novel emergence was unexpected based on the previous evidence that this pathogen, unlike Cryptococcus neoformans, was geographically restricted to tropical and subtropical regions throughout the world [5-8]. C. gattii can be classified into four discrete molecular types (Table 1), which represent cryptic species as no nuclear allelic exchange between groups has been observed [9,10]. This molecular classification is of fundamental importance. Of the four molecular types (VGI to VGIV), only one (VGII) is responsible for approximately 95% of all human and animal infections associated with the Vancouver Island outbreak and the subsequent expansion into the United States [1,3,10-14].

A central question from the analysis of the Vancouver Island outbreak relates to the origin of a novel genotype, VGIIa/major, which is responsible for the vast majority of all infections reported in British Columbia [1,15]. As in the sibling species C. neoformans, many C. gattii populations are predominantly composed of α mating type isolates, and, to date, all isolates related to the outbreak have been exclusively α [10]. A seminal finding by Lin and colleagues in 2005 was the discovery that α-α monokaryotic fruiting represents a novel mode of sexual reproduction (including meiosis) [16-18]. This finding, in combination with the discovery of an α/α VGIIa/major diploid isolate from Vancouver Island (RB59), and molecular comparisons between the VGIIa/major genotype and the less prevalent VGIIb/minor genotype that is also found in Australia, led to the hypothesis that same-sex mating may have produced the hypervirulent VGIIa/major genotype and may be responsible for ongoing production of infectious spores [10]. An alternative hypothesis is that opposite-sex mating, possibly in South America where α isolates similar in genotype have been discovered [19], gave rise to the outbreak isolate genotype [10]. Mating of C. neoformans and C. gattii can be stimulated by plants or plant
materials under laboratory conditions and may represent
environmental niches in which sexual reproduction may
occur [20,21]. In addition, the VGIIa/major subgroup
has been shown to be more fertile in comparison to the
VGIIb/minor subgroup [22]. While the origins of C. gattii
VGIIa/major in North America remain elusive, it is clear
that this emerging pathogen has invaded the United
States, and that, in addition, a new unique United States
genotype has arisen [11,12].

**Major recent advances**
In 2007 and 2008, the first reports of C. gattii in the
Pacific Northwest were published. The report of Upton
and colleagues [23] illustrated the first confirmed case of
the Vancouver Island outbreak VGIIa/major genotype in
the United States (2006) from a patient in Puget Sound,
Washington, and MacDougall and colleagues [3] dis-
covered related C. gattii VGII genotypes in the United
States in 2005, including one later recognized as a VGIIc
isolate. These studies prompted an increased surveil-
ance in Washington and Oregon, and retrospective studies
concluded that these were most likely the sentinel cases
in the region [23]. Subsequent to these reports, the
community has witnessed dozens of cases in the United
States, all occurring after 2006 [11,13,14], with mortality
in humans reaching levels over 25% (KA Marr and S West,
unpublished observations). Beyond mortality, these
infections are difficult to manage, prolonged, and a
cause of significant morbidity. In addition to human
cases, there has also been mortality among terrestrial
companion, agrarian, and wild animals in the United
States, as well as cases in wild marine mammals [11,12].

The dynamics of emerging outbreaks are often multi-
faceted, particularly in sexual pathogens, requiring in-
depth molecular typing methods, including multilocus
sequence typing (MLST), variable number of tandem
repeats typing, and epidemiological analysis [24-27].
From a molecular epidemiological perspective, there
were two recent findings of import regarding the specific
outbreak dynamics in the Pacific Northwest region of the
United States. The first finding is clear evidence that the
range of the hypervirulent VGIIa/major genotype has
expanded from British Columbia, Canada into the
United States [11]. The second development is the
discovery of a novel VGII genotype, VGIIc, found thus
far exclusively in Oregon, occurring in both humans and
other animals [11]. In addition to being geographically
restricted, recent evidence suggests that this genotype,
similar to VGIIa/major, is hypervirulent (EJB and JH,
unpublished observations).

Analysis of the current molecular data suggests that the
emergence in the United States may be more compli-
cated than originally thought. The apparent increase in
diversity seen in the US indicates that evolutionary forces
may be causing an increase in diversity. Phyloge-
netic analysis using maximum likelihood analysis at seven
MLST loci suggests that the novel VGIIc genotype is
distinct but closely related to the VGIIa/major genotype
when compared with several global VGII genotypes
(Figure 1) [10,11]. While closely related, this novel
 genotype has never been found on Vancouver Island,
even though this is one of the most sampled areas
globally. This genotype may have originated elsewhere,
or alternatively arose locally in Oregon via a genetic cross
or a mutational process. This line of evidence leaves
open the question of origin, and is a logical point of
investigation for future studies.

**Future directions**
While aspects of the dynamics of the evolution of VGII
in the United States remain unclear, recent population
genetic and molecular studies of other C. gattii popu-
lations have yielded fundamental insights into hypotheses
that should be explored in the US population. While
isolates of C. gattii have been shown to undergo a-α
mating under laboratory conditions [28], no α isolates
have been found in the Pacific Northwest outbreak,
making same-sex mating a more parsimonious explana-
tion for the origins of novel isolates and the production
of infectious spores, although no fruiting of C. gattii has
been reported under defined laboratory conditions. Two
recent studies from Australian populations, one clinical
and one environmental, provide evidence that same-
sex mating is occurring within these C. gattii and
C. neoformans populations [25,26], and similar popula-
tion-based approaches should be employed in
the Washington and Oregon C. gattii populations to
examine the possibility of ongoing recombination.
Another recent line of evidence suggests that mitochondrial inheritance and recombination could play significant roles in the evolution of \textit{C. gattii}\cite{29,30}. Mitochondrial recombination or exchange requires cell-cell fusion, and it is hypothesized that this type of event could also lead to other nuclear or plasmid genetic exchange. Detailed examination of mitochondrial genes in the outbreak population may help to illustrate levels of fusion and possible genetic recombination among this \(\alpha\) VGII population. Overall, both population genetic and molecular studies focused on the United States \textit{C. gattii} population will help establish the roles genetic exchange may have played in virulence acquisition and adaptive evolution to novel environments.

Abbreviation

MLST, multilocus sequence typing.

Competing interests

The authors declare that they have no competing interests.

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