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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 **a** 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

Table 1. *Cryptococcus gattii* molecular types

Species	Serotype	Molecular type	Description
C. gattii	B	VGI	Most common clinically, highly clonal
		VGII	Responsible for Pacific NW Vancouver Island outbreak and Northwestern United States outbreak
	C	VGIII	Highly fertile, more common in HIV+ patients
		VGIV	More common in HIV+ patients, rarely found

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] discovered related *C. gattii* VGII genotypes in the United States in 2005, including one later recognized as a VGIIc isolate. These studies prompted an increased surveillance 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].

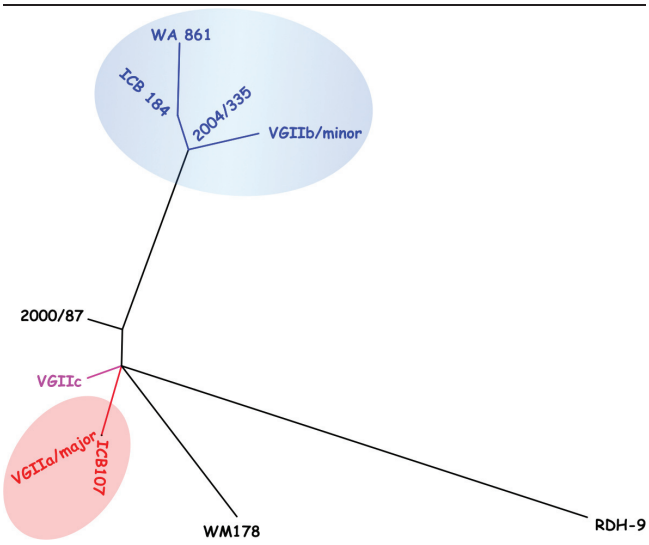
The dynamics of emerging outbreaks are often multifaceted, particularly in sexual pathogens, requiring in-depth molecular typing methods, including multilocus sequence typing (MLST), variable number of tandem repeats typing, and epidemiological analysis [24-27]. From a molecular epidemiological perspective, 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 complicated than originally thought. The apparent increase in diversity seen in the US indicates that evolutionary forces may be causing an increase in diversity. Phylogenetic 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* populations 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 **a** isolates have been found in the Pacific Northwest outbreak, making same-sex mating a more parsimonious explanation 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 population-based approaches should be employed in the Washington and Oregon *C. gattii* populations to examine the possibility of ongoing recombination.

Figure 1. Phylogeny of *C. gattii* VGII genotypes in the United States



This unrooted maximum likelihood tree is based on concatenated sequences from the following seven genes that are commonly used in MLST analysis of *C. gattii*: *IGS*, *TEF1*, *GPD1*, *LAC1*, *CAP10*, *PLB1*, and *MPD1*. All corresponding sequences and associated GenBank accession numbers can be found in [10,11]. The blue shaded group includes the VGIIb/minor and closely related genotypes, while the red shaded group indicates the VGIIa/major genotype. Pink coloration indicates the VGIIc novel genotype, which is closely related to the VGIIa/major genotype based on this analysis. Sequence alignments were conducted using the Clustal W software package (http://www.ebi.ac.uk/Tools/clustalw2/index.html), and the maximum likelihood tree was constructed using the PhyML software package (http://www.atgc-montpellier.fr/phyml/).

Another recent line of evidence suggests that mitochondrial inheritance and recombination could play significant roles in the evolution of *C. gattii* [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 α VGII population. Overall, both population genetic and molecular studies focused on the United States *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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