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Epidemiology of *Cryptococcus* and cryptococcosis in China

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ABSTRACT

Cryptococcosis is a significant invasive fungal infection with noteworthy morbidity and mortality, primarily caused by *Cryptococcus neoformans* and *Cryptococcus gattii*. In China, *C. neoformans* var. *grubii* (especially molecular type VNI) is the most common variety in the environment and responsible for the majority of cryptococcal infections. *C. gattii* infections are quite rare in China and the primary molecular type is VGI, which is closely related to *C. gattii* isolates in Australia. Interestingly, the majority of cryptococcosis in China were reported in the HIV-uninfected patients (especially immunocompetent hosts). This unique phenomenon may be attributed to multiple polymorphisms in the genes encoding mannose-binding lectin (MBL) and Fc-gamma receptor 2B (*FCGR2B*) in the Han population, the major ethnic group in China. Compared to immunocompromised patients, immunocompetent patients with cryptococcal meningitis often presented with more intense inflammatory responses and more severe neurological complications, but less fungal burdens and disseminated infection. The overall prognosis, which is independently associated with amphotericin B-based initial therapy, is similar between immunocompetent and immunocompromised patients. In addition, intrathecal administration of amphotericin B has been proved to be an effective adjunctive treatment for cryptococcosis in China.

1. Introduction

Cryptococcus is an important fungal pathogen causing life-threatening meningitis of significant morbidity and mortality. Among the 70 identified species of *Cryptococcus*, *Cryptococcus neoformans* and *Cryptococcus gattii* are the major causative agents of human cryptococcosis (Kurtzman et al., 2010). While *C. neoformans* is a major fungal pathogen causing infections in immunocompromised individuals such as AIDS patients, *C. gattii* has long been considered as the culprit causing cryptococcosis in immunocompetent hosts, recently highlighted in the outbreaks in Canada (British Columbia) and the United States Pacific Northwest (Datta et al., 2009; Galanis et al., 2010; Heitman et al., 2010; Sorrell, 2001). These two species are genetically related to each other, however, they vary in ecological niches, geographic distribution, and pathogenic characteristics. *C. neoformans* has a global distribution and is closely associated with avian habitats, while most *C. gattii* strains have been isolated from North America and Australia, and are commonly identified in Eucalyptus trees in the environment (Ellis and Pfeiffer, 1990). In addition, *C. gattii* infections often cause more frequent neurological complications than *C. neoformans*. It seems *C. gattii* is less sensitive to antifungal therapy than *C. neoformans*, therefore, more aggressive interventions are usually required in the treatment of *C. gattii* infections (Newton et al., 2002; Perfect et al., 2010; Speed and Dunt, 1995).

China is the third largest and the most populous (over 1.3 billion people) country in the world. The majority of people in China reside in the temperate and subtropical regions, a climate amenable for fungal growth and spread. In fact, the increasing number of immunocompromised population including patients with HIV infection, malignant tumors, organ or stem-cell transplantation, and autoimmune diseases during the past several decades in China has led to a rapid elevation of the incidence of invasive fungal infections such as cryptococcosis, which become a serious threat to the public health (Trey et al., 2011; Wang and Wang, 2010; Wu et al., 2011a; Wu and Lu, 2008; Yang et al., 2010). Unfortunately, relatively little is known about the diversity and epidemiology of the pathogenic *Cryptococcus* species complex in China. In this article, the ecology, epidemiology, and population genetics of *C. neoformans* and *C. gattii* in China will be reviewed and compared with data from other countries. In addition, the unique clinical features and epidemiology of cryptococcosis in China, and the Chinese physicians' management experience on cryptococcosis will also be discussed in the present review.

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2. Geographic distribution and environmental niches of *C. neoformans* and *C. gattii* in China

Human cryptococcal infections are thought to be acquired by inhalation of airborne spores or desiccated yeast cells from the environment (Lin and Heitman, 2006). Therefore, investigation of the geographic distribution and environmental niches of *C. neoformans* and *C. gattii* allows us to better understand the potential impacts of these environmental pathogenic fungi on human health.

In China, ecological studies of *C. neoformans* and *C. gattii* were initiated relatively late, and most studies were focused on the identification of cryptococcal strains from avian excreta. In 1993, Li et al. isolated 51 *C. neoformans* isolates from 36 samples of pigeon droppings in Nanjing city, East China (Li et al., 1993). Among them, 40 (78%) and 11 (22%) isolates were identified as serotype A and serotype AD, respectively, based on slide agglutination assays. Interestingly, both *C. neoformans* serotype A and serotype AD isolates were concurrently isolated from some samples. In 2000, Li et al. isolated 32 *C. neoformans* serotype A isolates from 144 samples of pigeon droppings in private and public pigeon shelters in Shanghai, China (Li et al., 2000). In 2011, five *C. neoformans* serotype A strains were identified from 143 samples of fresh domestic pigeons droppings in Beijing (Wu and Li, 2011). In 2012, a total of 358 *C. neoformans* isolates were isolated from 620 samples of avian excreta in 10 major cities from 20° to 50° North latitude (Li et al., 2012). Mycological tests of 101 isolates showed that all of them were *C. neoformans* serotype A and mating type α, which was consistent with the geographic distribution of cryptococcosis in China. The isolation rate of *C. neoformans* in the G2 region (30–40°N) was the highest (50%), followed by the G3 region (20–30°N) (29%) and the G1 region (40–50°N) (13%). Unfortunately, the molecular types of these environmental isolates were not determined. Recently, Lin et al. isolated 80 cryptococcal strains from 1372 avian fecal samples collected from 15 provinces in mainland China (Lin et al., 2013). All the 80 strains were identified as *C. neoformans* molecular type VNIc based on PCR fingerprinting, which is consistent with the major molecular type of clinical isolates in China (Chen et al., 2008).

Taken together, avian habitats were an important natural reservoir for *C. neoformans* in China, and *C. neoformans* serotype A strains (96%) were the predominant species in this environment. In total, 283 cryptococcal isolates were isolated from pigeon droppings in 16 provinces in mainland China (Fig. 1). The average isolation rate was 12.9%, which is significantly lower than that in other countries (Castanon-Olivares and Lopez-Martinez, 1994; Romeo et al., 2012). The significant difference of isolation rates of cryptococcal isolates between China and other countries may be attributed to differences in the isolation methods, sample quality, and some environmental factors. In addition, the geographic distribution of *C. neoformans* is associated with the longitude and climate. The southeastern region (tropical and subtropical regions with warm and wet climate) appears to be more appropriate for fungal growth and propagation compared with other regions in China. However, the ecological data from West China are currently not available, which highlights ecological and epidemiological studies in this area in the future.

Unlike *C. neoformans* that is mainly associated with pigeon droppings, *C. gattii* strains are mostly isolated from trees and decayed wood. For example, *C. gattii* strains, especially strains of the molecular type VGI, were closely associated with Eucalyptus trees in Australia and elsewhere (Chakrabarti et al., 1997; Ellis and Pfeiffer, 1990; Montenegro and Paula, 2000; Sorrell, 2001). *Eucalyptus camaldulensis* trees were imported from Australia to China and widely distributed between 17°N and 33°N, including Jiangxi, Guangdong, Guangxi, and Zhejiang provinces. Currently, there is only one report on *C. gattii* ecology in China. Li et al. collected 819 samples of flower, bud, leaf, decayed wood, and bark from 40 *E. camaldulensis* trees in the Jiangxi province, China (Li et al., 2012). However, no *C. gattii* strains were isolated from these tree samples, which may be due to the physiological adaptation of *E. camaldulensis* to the changes of climate or ecological environment. Molecular identification methods, which are more sensitive than culture-dependent approaches, may be useful in ecological studies of *C. gattii* in China. In addition, identification and characterization of other ecological niches of *C. gattii* should also be

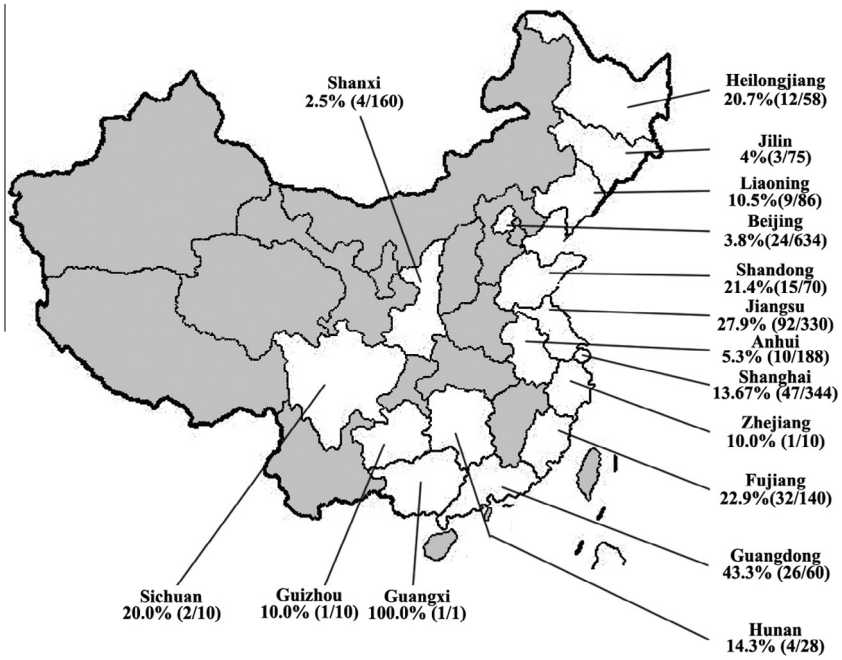

Fig. 1. Summary of ecological studies on *C. neoformans* conducted in mainland China between 1993 and 2013. A total of 283 cryptococcal strains were isolated from 2194 avian fecal samples from 16 provinces in China (Li et al., 1993, 2012; Li et al., 2000; Lin et al., 2013; Wu et al., 2011b). The positive rate of isolation was variable in different regions, which was relatively higher in Southeast regions. The shadowed areas represented those provinces where no ecological data about *C. neoformans* were available.

conducted. For example, several plant species such as *Tamarindus indica*, *Pithecolobium dulce*, *Syzygium cumini*, and *Manilkara hexandra* have been demonstrated to be more appropriate environmental reservoirs for *C. gattii* than Eucalyptus in India, (Grover et al., 2007; Randhawa et al., 2006, 2008).

3. Characteristics of clinical isolates of *C. neoformans* and *C. gattii* in China

C. neoformans var. *grubii* (serotype A) is the most common causative agent of cryptococcal meningitis worldwide (80%), especially in AIDS patients. *C. gattii* infections, which have been mainly reported in Australia and America, are much less prevalent than infections caused by *C. neoformans* (Chayakulkeeree and Perfect, 2006). In Asia, most population genetic studies of the *C. neoformans* species complex were from Thailand and India. Since the 1990s, several studies have been performed to explore the epidemiology of clinical isolates of *C. neoformans* and *C. gattii* in China based on serological and/or molecular tests.

Most clinical isolates of the pathogenic *C. neoformans* species complex in China were identified as serotype A and B strains, while serotype D and AD strains account for a small percentage, which is consistent with the results of ecological studies. In 1987, Li et al. conducted the first serotyping study of clinical cryptococcal isolates from 9 regions in mainland China, and identified 49 *C. neoformans* serotype A and 9 *C. gattii* serotype B strains (Li et al., 1987). The authors concluded that serotype A and B strains were the major causative agents of cryptococcosis in China, and even in Asia, which was supported by many subsequent studies. For example, Li et al. identified 17 serotype A (89.5%) and 2 serotype B (10.5%) strains among 19 cryptococcal clinical strains isolated from Shanghai and Nanjing cities in 1993 (Li et al., 1993). Another study reported 20 serotype A (95.2%) and 1 serotype B strains (4.8%) isolated from patients in Taiwan, and all these isolates were mating type alpha (19/19) (Hsu et al., 1994).

Several studies have characterized the molecular types of clinical cryptococcal isolates from China. Based on PCR-fingerprinting and multilocus sequence typing (MLST), Chen et al. identified 120 (93%) VNI (*C. neoformans* serotype A) and 9 (7%) VGI (*C. gattii* serotype B) strains among a collection of 129 clinical cryptococcal isolates from sixteen provinces in mainland China (Chen et al., 2008). Phylogenetic analyses grouped all VNI strains into a unique cluster (the VNIc cluster) with environmental strains reported in an ecological study in China (Lin et al., 2013). *C. neoformans* usually causes cryptococcosis in immunocompromised patients (Heitman et al., 2010). However, most VNI isolates (84, 70%) in this study were isolated from patients with no significant immunodeficiency (Chen et al., 2008). Feng et al. also reported that the predominant molecular type was VNI among clinical cryptococcal isolates from China (Feng et al., 2008). The authors identified 103 VNI isolates (89.5%) from 115 clinical isolates in mainland China. Except one strain that was mating type a (serotype D, molecular type VNIV), other strains were identified to be mating type alpha. In addition, most strains (75/78, 96.2%) were isolated from HIV-uninfected patients. Among the 115 clinical cryptococcal isolates, 9 (7.8%) strains were identified to be *C. gattii*, which were isolated from East (subtropical zone) and South China (tropical zone). The 9 *C. gattii* strains were further classified into molecular types VGI (8, 7.0%) and VGII (1, 0.9%). Subsequently, Chen et al. compared the genotypic distribution of cryptococcal isolates between HIV-positive and HIV-negative patients in southeast China using PCR fingerprinting and DNA sequencing of the internal transcribed spacers of rDNA (ITS region) and the capsule-associated gene (*CAP59*) (Chen et al., 2010). A total of 109 *C. neoformans* and 12 *C. gattii* strains were isolated from cryptococcosis patients. All the 12 *C. gattii* (11 VGI and 1 VGII molecular types) were isolated from HIV-uninfected patients. Among the 109 *C. neoformans* isolates, 89 (82%) and 20 (18%) were isolated from HIV-uninfected and HIV-infected patients, respectively. In addition, the molecular type VNI predominated in the *C. neoformans* clinical isolates (79, 72%) (Chen et al., 2010). In 2010, Liaw et al. characterized 100 clinical cryptococcal strains from a teaching hospital in Taiwan (Liaw et al., 2010). Based on PCR fingerprinting, 99 strains were identified as *C. neoformans* serotype A (98 VNI and 1 VNII molecular types) and only one was identified as *C. gattii* serotype B (molecular type VGI). Tseng and his colleagues analyzed the molecular types of 219 isolates from 20 hospitals throughout Taiwan during 1997–2010 (Tseng et al., 2013). They identified 210 (95.9%) *C. neoformans* isolates including 206 VNI and 4 VNII isolates, and 9 *C. gattii* isolates including 3 VGI and 6 VGII isolates. The VNI molecular type predominated in both HIV-infected (98.1%, 53/54) and HIV-uninfected patients (91.9%, 137/149) and *C. gattii* was more commonly isolated from HIV-uninfected patients (88.9%, 8/9) than HIV-infected patients in Taiwan, which is consistent with the results in mainland China.

Multilocus microsatellite typing (MLMT) has been successfully used to discriminate a number of pathogenic fungi (De-Valk et al., 2007; Illnait-Zaragozi et al., 2010). MLMT in which highly polymorphic microsatellite markers are used as genotyping markers exhibited significant higher discriminatory power than housekeeping genes-based MLST, therefore, MLMT can discriminate closely related strains that may be genetically identical based on MLST. Using MLMT with 9 serotype A-specific microsatellite markers, Zhu et al. characterized 43 *C. neoformans* serotype A strains from 6 Chinese cities in 2009 (Zhu et al., 2009). Among the 43 serotype A strains, genotype MLMT-17 predominated in both clinical (86.67%, 26/30) and environmental isolates (70%, 7/10), while other genotypes including MLMT-14, 16, 29, 39 and 40 were rare. The predominant genotype MLMT-17 has also been reported in another two studies on *C. neoformans* clinical isolates from Guizhou (85.7%), Guangxi (96.7%), and Jilin (91.4%) provinces in mainland China (Gao and Wang, 2013; Kang et al., 2013). The genotype MLMT-17 of *C. neoformans* is likely the major genotype in East Asia, which is significantly different from the genotypic distribution of *C. neoformans* in other countries (Hanafy et al., 2008). For example, MLMT-22 was the predominant genotype in Africa and Europe, while genotypes MLMT-13 and MLMT-22 were common in South America. Based on MLMT analysis, Pan et al. further explored the genetic diversity of *C. neoformans* serotype A isolates in China (Pan et al., 2011, 2012). The 116 *C. neoformans* serotype A strains isolated from patients in 15 provinces in Southeast and Central China were classified into 62 MLMT genotypes and grouped into three microsatellite complexes (MCs). Interestingly, most strains ($n = 103$) were grouped into MC2. By contrast, MC3, MC16 and MC8 predominated in *C. neoformans* serotype A clinical isolates from India, Japan, and other Southeast Asian countries (i.e., Indonesia and Thailand). In addition, most MC2 strains were isolated from HIV-uninfected patients, whereas MC8 strains were common in HIV-infected patients (Pan et al., 2012).

Taken together, *C. neoformans* strains of serotype A, molecular type VNI, and mating type α predominate in both immunocompetent and immunocompromised patients in China (See Table 1). Interestingly, the majority of those strains were isolated from HIV-uninfected patients, which is consistent with the results from other Asian countries such as Vietnam but inconsistent with the reports from other regions of the world (Chau et al., 2010; Dromer et al., 1996; Hajjeh et al., 1999; Moosa and Coovadia, 1997). Nevertheless, local geographic differences of genotypic structure for *C. neoformans* var. *grubii* were significant in Asia, which might be due to different founder effects and/or environmental factors (including both ecological and host environment)

Table 1
Clinical *Cryptococcus* isolates identified in various regions in China.

Year	Region	No. of isolates	Method	Serotype, molecular type, and genotype	Reference
1987	9 regions	58	Slide agglutination	A (49), B (9)	Li et al. (1987)
1993	Shanghai & Nanjing	19		A (17), B (2)	Li et al. (1993)
1994	Taiwan	21	Slide agglutination	A (20), B(1)	Hsu et al. (1994)
2008	16 provinces	129	RFLP, MLST	A, VNI (120); B, VGI (9)	Chen et al. (2008)
2008	Six regions	115	RFLP, MLST	A, VNI (103); AD, VNIII (2); D, VNIV (1); B, VGI (8), VGII (1)	Feng et al. (2008))
2009	6 provinces	43[a]	MLMT	A; MLMT-14(1), -16(1), 17(36), 29(1), 34(1), 39(1), 40(2)	Zhu et al. (2009)
2010	10 provinces	109	RFLP	VNI(79), VNII(15), VNIII(2); VGI (11), VGII (1)	Chen et al. (2010)
2010	Taiwan	100	RFLP	A, VNI(98), VNII(1); B, VGI(1)	Liaw et al. (2010))
2013	Taiwan	219	RFLP	A, VNI(206), VNII(4); B, VGI (3), VGII(6)	Tseng et al. (2013))
2013	Guizhou & Guangxi	58	MLMT	MLMT-9, 17, 26, 40, 41	Kang et al. (2013)
2013	Jilin	58	MLMT	9(1), 17(53), 26(1), 40(2), 41(1)	Gao and Wang (2013)
2013	15 provinces	116	MLMT	MC2(103), MC3(3), MC(12)	Pan et al. (2011, 2012)

[a] Including 10 environmental isolates of *C. neoformans*.

(Jain et al., 2005; Pan et al., 2012). Other molecular types of *C. neoformans* such as VNIII (serotype AD) and VNIV (serotype D) were occasionally isolated from Chinese patients and the isolation rate of these two serotypes in China were lower than that in Europe and South America (Dromer et al., 1994; Lemmer et al., 2004; Trilles et al., 2008; Viviani et al., 2006). In addition, *C. neoformans* strains in China exhibited relatively low genetic diversity based on AFLP, MLST, and MLMT analyses, which is consistent with reports from other Asian countries such as India and Thailand, and the genetic diversity of Asian strains was generally lower than strains isolated from other regions of the world (Barreto-de-Oliveira et al., 2004; Jain et al., 2005; Meyer et al., 1999; Simwami et al., 2011; Sriburee et al., 2004).

The incidence of *C. gattii* infection (1–11%), which mainly occurred in the subtropical and tropical areas (Eastern and Southern China) such as Guangdong, Zhejiang, Shanghai, and Taiwan, was significant lower than that of *C. neoformans* infection (See Table 1). The low incidence of *C. gattii* infection was close to that in other Asian countries and Europe, but lower than the average global incidence of *C. gattii* infection (20%) (Heitman et al., 2010). The major *C. gattii* molecular type in China was VGI, accounting for 78.9% (30/38) of clinical *C. gattii* isolates. The isolate S8012 (ATCC56992 or CBS7229) was initially reported as *C. neoformans* var. *shanghaiensis* by Liao et al. in 1980s, but has been molecularly identified to be VGI recently (Chen et al., 2011; Liao et al., 1983). Globally, *C. gattii* VGI isolates predominate in clinical isolates in Australia and Papua New Guinea (Campbell et al., 2005; Heitman et al., 2010; Sorrell et al., 1996). Based on MLST and phylogenetic analyses, it has been confirmed that *C. gattii* VGI isolates from China were closely associated with *C. gattii* strains from Australia, suggesting that the potential origin and distribution of *C. gattii* VGI strains in China (Chen et al., 2011; Feng et al., 2008). Since 1999, hypervirulent strains of *C. gattii* VGII molecular type have caused several outbreaks in Canada (British Columbia) and the Pacific Northwest United States (Washington and Oregon) (Datta et al., 2009). The *C. gattii* VGII strains had been proved to be the major pathogen causing cryptococcosis in immunocompetent patients in the world (Heitman et al., 2010). In 2007, Okamoto reported the first cryptococcosis case caused by a VGIIa strain in Japan and Asia (Okamoto et al., 2010). This strain was identical to the isolate R265 (the Vancouver Island outbreak major genotype strain) based on MLST, suggesting that this hypervirulent strain may spread to Asia. In China, a *C. gattii* VGIIb strain (XH91) that is closely related to the R272 strain (Vancouver Island outbreak minor genotype strain) was recently isolated from a patient in Guangdong, China (Feng et al., 2008, 2010). While most *C. gattii* infections in China were caused by the VGI molecular type, isolation of the VGIIb strain from a Chinese patient suggests that cryptococcosis caused by *C. gattii* VGII molecular type also exist in China.

4. Epidemiology and molecular genetics of clinical isolates of *C. neoformans* and *C. gattii* in China

Since the first cryptococcosis case was described by Buss and Buschke in 1894, research on cryptococcosis has a 120-year history (Buschke, 1895; Busse, 1894). Due to the global AIDS pandemic and wide use of chemotherapy and immunosuppressants, the incidence of cryptococcal infections (especially meningoencephalitis) has significantly increased in the past decades. It has been estimated that the global burden of cryptococcal meningitis was approximately 957,900 cases per year, resulting in 624,700 deaths annually (Park et al., 2009). Due to distinct HIV endemic status and geographic and ethnic factors, the incidence of cryptococcosis varies in different countries and regions (Chen et al., 2000; Friedman et al., 2005; McCarthy et al., 2006; Mirza et al., 2003). For example, the annual incidence of cryptococcosis was 6.6 cases per million person in Australia, 15.6 per 100,000 person in Gauteng Province, South Africa (Chen et al., 2000; McCarthy et al., 2006). Studies on the prevalence and epidemiology of cryptococcosis in China have been very limited. A recent systematic review has reported a total of 8769 cases of cryptococcosis in mainland China between 1985 and 2010 (Chen et al., 2012). However, the incidence of cryptococcosis in mainland China may be much higher than reported in this review because of missed and misdiagnosed cases and publication bias. In 2011, Chen et al. conducted a population-based epidemiological study of cryptococcal meningitis in Taiwan from 2000 to 2007, and reported an annual incidence of 4.7 cases per million person (Chen and Lai, 2011). In general, the number of reported cryptococcosis in China has been increasing over recent decades (Wu et al., 2011a; Chen et al., 2012; Zhu et al., 2010).

Cryptococcosis has been defined as an opportunistic fungal infection because it mainly occurs in immunocompromised populations such as patients with AIDS, organ transplantation recipients, patients with autoimmune diseases. Studies from the U.S., France, and Thailand reported a relatively small proportion (17–35%) of cryptococcosis occurring in patients with apparently healthy condition (Kiertiburanakul et al., 2006; Pappas et al., 2001). However, a significantly high proportion of cryptococcosis were reported in immunocompetent individuals in China (See Table 2) (Chen et al., 2008, 2012; Chen and Lai, 2011; Lu et al., 1999; Lui et al., 2006; Shih et al., 2000; Tseng et al., 2013; Zhu et al., 2010). For example, among the 154 cryptococcal meningitis cases reported at Huashan Hospital, Shanghai, 103 (66.9%) cases were identified in immunocompetent individuals (Zhu et al., 2010). Similarly, 68% of cryptococcal meningitis cases were reported in immunocompetent individuals in another study performed at Changzheng Hospital, Shanghai (Chen et al., 2008). Several hospital-based studies conducted in Taiwan reported that 55.3% (Shih et al., 2000), 60.6% (Lu et al., 1999), 25% (Liao et al., 2012), and 43.5% (Lui et al., 2006) of cryptococcosis cases occurred

Table 2
Demographic features and underlying diseases of cryptococcosis patients with or without HIV infection from hospital-based studies.

Parameter	Without HIV infection			With HIV infection		
	Lu et al. (1999)	Shih et al. (2000)	Zhu et al. (2010)	Lui et al. (2006)	Liao et al. (2012)	Tseng et al. (2013)
Publication year	1999	2000	2010	2006	2012	2013
Region	Taiwan	Taiwan	Shanghai	Hong Kong	Taiwan	Taiwan
Period	1986–1997	1977–1996	1997–2007	1995–2005	1995–2009	1997–2010
No. of Patients	71	94	154	46	72	219
Sex						
% (male/total)	64.8% (46/71)	52.8% (59/94)	61.0% (94/154)	60.9% (28/46)	75% (54/72)	67.6% (148/219)
Age (yr)[a]	(15, 83)	37.7 ± 17.5 (4, 83)	38.5 (9, 75)	50.0 ± 3.1		52.5 ± 18.2 (12, 94)
Predisposing factors						
HIV/AIDS				19.6% (9/46)	26.4% (19/72)	24.7% (54/219)
Corticosteroids and immunosuppressives			13.6% (21/154)	21.7% (10/46)	18.1% (13/72)	
Autoimmune diseases	7.0% (5/71)	10.6% (10/94)	11.0% (17/154)	10.9% (5/46)	6.9% (5/72)	5.0% (11/219)
HBV						21.0% (46/219)
Liver cirrhosis	7.0% (5/71)	6.4% (6/94)	9.7% (15/154)	8.7% (4/46)	16.7% (12/72)	14.2% (31/219)
Diabetes mellitus	5.6% (4/71)	8.5%(8/94)	9.1% (14/154)	19.6% (9/46)	16.7% (12/72)	18.3% (40/219)
Tuberculosis	5.6% (4/71)					2.7% (6/219)
Immunosuppression			8.4% (13/154)			
Chronic kidney disease			7.1% (11/154)	4.3% (2/46)	12.5% (9/72)	9.6% (21/219)
Splenectomy			1.3% (2/154)			
Solid malignancy	5.6% (4/71)	6.4% (6/94)	0.7% (1/154)	10.9% (5/46)	11.1% (8/72)	14.2% (31/219)
Hematologic malignancy		11.7% (11/94)	0.7% (1/154)			5.9% (13/219)
Transplantation		6.4% (6/94)	0.7% (1/154)		2.8% (2/72)	1.8% (4/219)
Idiopathic CD4 lymphocytopenia		31.9% (30/94)	12.5% (9/72)	2.2% (1/46)		1.4% (3/219)
Without predisposing factors	60.6% (43/71)	55.3% (52/94)	66.9% (103/154)	43.5% (20/46)	25% (18/72)	10.5% (23/219)

[a] These studies displayed different format of age in the papers.

in immunocompetent individuals. In addition, a population-based study reported 61.9% of cryptococcosis cases were from immunocompetent individuals in Taiwan (Chen and Lai, 2011). However, a relatively small proportion (10.5%) of cryptococcosis in immonocompetent hosts has been reported in a study conducted in 20 hospitals in Taiwan (Tseng et al., 2013), which was likely due to distinct admission rates and preponderant department bias in different hospitals. Furthermore, studies from other Asian countries where Chinese is the predominant ethnic group reported similar results. For example, it has been shown that 96% of cryptococcosis in Singapore were from immunocompetent individuals and only 6.7% of cryptococcosis were identified in immunocompromised patients in Malaysia (Richardson et al., 1976; Tjia et al., 1985). Similarly, in a study on HIV-uninfected patients with cryptococcal meningitis in Vietnam, 81% of them (57 patients) had no clear predisposing factor (Chau et al., 2010). Taken together, these reports suggest that cryptococcosis predominantly occurs in immunocompetent hosts in the Chinese ethnic population that might be more susceptible to cryptococcal infections than other ethnic groups.

Among patients with underlying conditions, HIV infection has remained the most common risk factor for cryptococcosis in China (Chen and Lai, 2011; Lui et al., 2006; Tseng et al., 2013; Chen et al., 2012). Studies from mainland China, Taiwan, and Hong Kong showed that the prevalence of cryptococcosis in HIV/AIDS patients ranged from 12.9% to 24.7%, which is significantly lower than that in other regions of the world (Banerjee, 2005; Chen et al., 2000; Dromer et al., 1996, 2007; Friedman et al., 2005; Jongwutiwes et al., 2008). The relatively low prevalence of cryptococcosis in HIV/AIDS patients in mainland China, Taiwan, and Hong Kong may be attributed to several reasons. Firstly, various AIDS control measures including national surveillance and civil campaigns have significantly slowed the spread of HIV in China (Wang and Wang, 2010). At the end of 2009, only 740,000 HIV/AIDS cases were reported in China. Secondly, invasive fungal infections were more common in patients with low CD4+ T cells and the incidence of cryptococcosis among HIV-infected patients declined rapidly due to the wide use of fluconazole and the introduction of HAART in the past decade (Friedman et al., 2005; Kaplan et al., 2000; Shen et al., 2007). Thirdly, the majority of HIV-infection cases in China were reported in less developed areas such as Yunnan, Guangxi, and Henan (Wang and Wang, 2010). Poor hygiene and medical services limited the outpatient rate, and delay the diagnosis and treatment of cryptococcal meningitis in many patients. The relationship between HIV status and cryptococcosis incidence remained to be further illuminated in China. Other than HIV infections, several conditions such as autoimmune diseases, immunosuppressant utilization, malignancy, diabetes, and organ transplantation were also important predisposing factors for cryptococcosis (See Table 2). It was noteworthy that 14% and 12% of cryptococcosis patients in China had tuberculosis and liver diseases, respectively, which is consistent with the high prevalence of Mycobacterium and HBV infection in China (Chen et al., 2012).

Overall, HIV-uninfected patients were the majority of the susceptible population to cryptococcosis in China, in contrast to most other countries. In order to investigate the potential genetic predisposition to cryptococcosis in the Chinese population, Ou et al. performed a case-controlled study about the association between mannose-binding lectin (MBL) polymorphisms and the development of cryptococcal meningitis in non-HIV patients (Ou et al., 2011). MBL, an important member of pathogen recognition receptors (PRRs), is essential for activating host innate immunity. The authors found that the genotypes underlying deficient MBL production was associated with cryptococcal meningitis ($P = 0.039$, OR = 2.09), particularly, in immunocompetent patients ($P = 0.028$, OR = 2.51), suggesting that MBL-deficiency is a genetic predisposition to cryptococcal meningitis in the Chinese Han ethnicity. However, the study from Australia did not identify a statistically significant association between MBL deficiency and cryptococcosis in immunocompetent hosts, which might be attributed to the small sample size (36 patients), characteristics of pathogen constitution, and ethnic factors (Eisen et al., 2008). In 2012, the association between FCGR (Fc-gamma receptors FcγRs coding genes) polymorphisms and cryptococcal meningitis was analyzed in 117 patients and 190 healthy controls by multiplex SNaPshot genotyping (Hu et al., 2012). The investigation revealed that the *FCGR2B* 232I/T genotype was associated with cryptococcal meningitis in HIV-uninfected individuals ($P = 0.016$, OR = 0.542), while no

Table 3
Clinical features of non-HIV-associated cryptococcosis in immunocompetent and immunocompromised hosts in three studies from China.

Parameter	Zhu et al. (2010) Mean/% (N/total or range)			Shih et al. (2000) Mean/% (N/total or range)			Lui et al. (2006) Mean ± SD/% (N/total)	
	Predisposed	Normal hosts	*P*	Predisposed	Normal hosts	*P*	Predisposed	Normal hosts
Number of patients	51	103		30	64		8	16
Age (yr)	48 (14, 67)	35 (9, 75)	0.0001					
Time to diagnosis (month)	30 (1, 124)	40 (6, 2890)	0.0062	14 (1900)	29 (1300)	0.015	15.3 ± 6.9	34.4 ± 7.7
Symptoms								
Fever >39 °C	41.2% (21/51)	16.5% (17/103)	0.001					
Coma	7.8% (4/51)	11.7% (12/103)	0.466					
Seizure	17.7% (9/51)	34.0% (35/103)	0.035					
Cerebral herniation	7.8% (4/51)	25.2% (26/103)	0.01					
Meningeal sign				46.7% (14/30)	79.7% (51/64)	0.002		
Fungemia				23% (7/30)	9% (6/64)	0.07	25% (2/8)	0 (0/16)
Lymphocyte in blood				1000 (59, 10,423)	1500 (200, 9219)	0.02		
CSF								
WBC	63 (0, 756)	100 (0, 1030)	0.0611	20 (0, 306)	77 (0, 1098)	0.004	53.9 ± 36.8	108.4 ± 25.1
Cryptococcal antigen	1280 (10, >1280)	1280 (1, >1280)	0.7805	512 (8, 16,348)	192 (0, 16,348)	NS	643.4 ± 576.3	810.0 ± 354.8
Cranial imaging								
Parenchymal lesions (MRI)	88.9% (16/18)	61.7% (29/47)	0.034					
Hydrocephalus (MRI/CT)	7.4% (2/27)	20.9% (14/67)	0.1	23% (4/17)	49% (23/47)	0.07	12.5% (1/8)	31.3% (5/16)
Meningeal enhancement (CT)				23.5% (4/17)	57.4% (27/47)	0.02		
Hypodense lesion (CT)				0% (0/17)	23.7% (9/47)	0.05		
Surgical procedure	5.9% (3/51)	23.3% (24/103)	0.007					

significant association was found between *FCGR2A*, *FCGR3A*, and *FCGR3B* genotypes and cryptococcal meningitis. The results suggest that FcγRIIB polymorphisms are another important genetic factor contributing to cryptococcal meningitis in HIV-uninfected Chinese individuals. In addition to MBL and FCGR, genetic variations of other PRRs such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs) might also be associated with the development of cryptococcal infections (unpublished data) (Zhu et al., 2014). Identification of other genes involved in cryptococcal infections may allow us to develop more effective therapy for the treatment of cryptococcosis.

5. Clinical manifestations and management of cryptococcosis in China

The central nervous system (CNS) and lungs are the most frequently involved organs in cryptococcosis. According to the latest literature review of cryptococcosis in mainland China (1985–2010), CNS and pulmonary infections occurred in 83.4% (7315/8769) and 13.0% (1142/8769) of cryptococcosis (Chen et al., 2012). High prevalence of CNS involvement in cryptococcosis patients has also been reported in Taiwan (58.9%) and Hong Kong (CNS, 67.4%) (Lui et al., 2006; Tseng et al., 2013). In addition, a study reported that *C. gattii* infections presented more often with CNS involvement (100%, $n = 9$) than *C. neoformans* (57.1%, $n = 210$) in China, which is different from the reports in other countries (Speed and Dunt, 1995; Tseng et al., 2013). However, this phenomenon should be confirmed by studies of larger sample size.

Clinical features of cryptococcosis vary in different susceptible populations, presumably depending on initial pathogen load, host immune status, and geographic or ethnic factors. As mentioned above, cryptococcal infections in China mainly occurred in non-HIV individuals, especially otherwise healthy hosts. Zhu et al. analyzed the clinical features of cryptococcal meningitis in immunocompetent hosts in a Chinese tertiary care hospital (1997–2007, See Table 3) (Zhu et al., 2010). Compared to immunocompromised hosts, previously healthy patients were ~10 years younger and their time to diagnosis took longer. Additionally, immunocompetent patients exhibited more severe neurological complications such as cerebral herniation, coma, seizure, and hydrocephalus than immunocomprised patients, and thus experienced more surgical shunt procedures. However, high fever and parenchymal lesions in brains were more common in immunocompromised patients. Similar differences in clinical manifestations between these two groups of patients have also been reported in Taiwan and Hong Kong (See Table 3) (Lui et al., 2006; Shih et al., 2000). Immunocompetent patients often presented with longer symptom durations and typical meningitis (meningeal signs and CT scan findings), which is likely due to more robust host immune responses (higher CSF cell counts and systemic lymphocyte counts). Conversely, immunocompromised patients tended to have higher fungal burden and higher incidence of disseminated infection (fungemia) (Shih et al., 2000). For example, HIV-infected patients often had higher serum cryptococcal antigen titers ($\geqslant$512), higher positive rate of fungal cultures from CSF and blood, and more severe symptoms or even systemic dissemination of infections than non-HIV hosts (Liao et al., 2012; Tseng et al., 2013; Yu et al., 2012). Similarly, it has been reported that cryptococcal meningitis patients with HBV infection exhibited higher positive CSF culture, more extraneural involvements, and lower inflammatory responses than HBV-negative patients (Zhong et al., 2014). Despite lack of serious underlying diseases, immunocompetent individuals with cryptococcosis exhibited similar treatment responses and prognosis as immunocompromised patients. It has been reported that no statistical differences in the overall mortality at one year were identified between these two groups of cryptococcosis patients ($P = 0.69$) (Zhu et al., 2010). In this retrospective study, coma, cerebral herniation, non-amphotericin B-based initial therapy, and delayed diagnosis (>120 days) were identified as independent risk factors of mortality of cryptococcosis patients. Other studies also reported that initial consciousness level, hydrocephalus, high CSF antigen titers, and underlying diseases such as malignancy, active HBV infection, and liver cirrhosis were poor prognostic factors for non-HIV-infected cryptococcosis patients (Liao et al., 2012; Lu et al., 1999; Lui et al., 2006; Shih et al., 2000; Zhong et al., 2014).

The incidence of pulmonary cryptococcosis in China and other countries have significantly increased in the past decades

(Galanis et al., 2010; Zhang, 2009). However, decrease of misdiagnosis due to the wide use of serological testing and improved radiological examination may lead to the increased incidence of pulmonary cryptococcosis. Similar to CNS involvement, pulmonary cryptococcosis mainly occurred in the HIV-uninfected population in China, and the majority of patients were immunocompetent. According to a review by Zhang, 69.7% (404/580) of pulmonary cryptococcosis cases had no underlying conditions and only 3.6% (21/580) pulmonary cryptococcosis patients had HIV infection (Zhang, 2009), which is consistent with several other single-center reports (Chang et al., 2006; Yu et al., 2012; Zhang et al., 2012). The most common symptoms of pulmonary cryptococcosis are cough, expectoration, chest tightness, fever, and other nonspecific manifestations. The severity depends mainly on host immune status. For example, a large proportion of immunocompetent patients (24–85.4%) were asymptomatic or had mild symptoms compared to immunocompromised patients (Ye et al., 2012; Yu et al., 2012; Zhang et al., 2012). For these reasons, some pulmonary cryptococcosis cases may be misdiagnosed as other lung diseases such as cancer and pneumonia. In addition, diagnosis of some cases of cryptococcal infection was only confirmed through incidental radiologic and/or histopathological examinations. The most frequent radiological signs of pulmonary cryptococcosis were single or multiple nodular masses and patchy consolidations mainly in subpleural areas and lower lung fields. The lesion patterns were primarily associated with host immune status. Generally, extensive pulmonary involvement and pneumonic infiltrates such as cavitation within nodules and parenchymal consolidation were significantly more common in immunocomprised patients than immunocompetent patients (Chang et al., 2006; Zhang et al., 2012). Histopathologically, initial pulmonary lesions gradually developed into granulomas in immunocompetent patients, but they frequently disseminate in the lungs in immunocomprised patients. In general, pulmonary cryptococcosis in HIV-uninfected patients has a good prognosis due to early diagnosis and appropriate intervention. The majority of asymptomatic cryptococcosis in immunocompetent undergo spontaneous remission even without antifungal therapy.

In long-term research and practice, Chinese doctors gradually developed their own experiences on the management of cryptococcal infections, especially in HIV-uninfected patients. In a study by Zhu et al., non-amphotericin B (AmB)-based initial therapy had been identified as the strongest independent factor associated with poor prognosis of cryptococcosis (Zhu et al., 2010). Compared to other antifungal drugs used in initial therapy, initial therapy with AmB resulted in higher rates of overall responses at either week 2 or 10, and significantly lower overall mortality at one year. In addition, it has been reported that intrathecal administration of AmB, a special treatment for CNS cryptococcosis, was an effective adjunctive treatment for many cryptococcosis patients in China, although it is no longer used in most countries and not recommended by the guidelines (Perfect et al., 2010). In the protocol of intrathecal AmB administration, AmB was diluted in sterile water, mixed with 4–5 ml auto-CSF and 1–2 mg dexamethasone, and infused slowly via lumbar injection (increasing from 0.1 to 1 mg, twice to three times per week). The regimen was continued for about 8 weeks until CSF cultures were negative. Among the 40 CNS cryptococcosis patients who received a combination of intrathecal and intravenous injection of AmB in Shanghai Changzheng Hospital, 39 patients were cured without recurrence (Yao et al., 2005). Chen et al. also reported that the combination of intrathecal and intravenous injection of AmB cured 683 (33%) of 1385 CNS cryptococcosis patients in China (Chen et al., 2012). As an adjunctive therapy, intrathecal injection of AmB enhanced the overall effectiveness from 72% to 74% ($P < 0.05$) and decreased the mortality from 28% to 26% ($P < 0.05$), likely due to its role in elevating the concentration of AmB in CSF and reducing the intracranial pressure. However, strict aseptic manipulation and close observation of adverse drug reaction such as nosocomial infection, retention of urine, and irritated cerebrospinal meningitis were important for the success of this special treatment protocol. An expert-guided protocol of two-phase therapy has been established for the treatment of cryptococcal infections in HIV-uninfected patients in China (Weng et al., 2010; Yao et al., 2005). For CNS infections, patients receive AmB both intravenously (0.5–1 mg/kg d) and intrathecally, and 5-Fluorocytosine for at least 8 weeks in the induction stage, followed by fluconazole or itraconazole therapy for at least 12 weeks in the maintenance stage. In this protocol, AmB administration in the induction stage was much longer than the 2 weeks recommended in the guidelines by the Infectious Diseases Society of America (Perfect et al., 2010). Based on Chinese physicians' experience, patients with cryptococcal meningoencephalitis usually need an average of 8 weeks of initial AmB therapy to obtain negative results of CSF culture (Yao et al., 2005). However, the efficacy of these two regiments should be evaluated and compared to further improve the treatment of cryptococcosis based on large-scale clinical trials.

6. Conclusion

Over the past decades, significant progress has been made in the ecological distribution, population genetics, molecular epidemiology, and clinical features of *Cryptococcus* and cryptococcosis in China. However, there are still many unanswered questions. First, the ecological characteristics of *C. neoformans* in western and northern regions, and the environmental niche of *C. gattii* in China remain unclear. In addition, the significance of environmental strains of *Cryptococcus* spp. in human infections needs to be addressed. Second, most of the molecular and epidemiological data originated from single-center retrospective studies, which cannot reflect the comprehensive prevalence and fungal burden of cryptococcosis in China. Therefore, effective nationwide surveillance of invasive fungal infections is important for this purpose. Questions such as the relationship between specific genotypes and phenotypes, the genetic diversity of Chinese clinical isolates and their association with global strains, and why immunocompetent individuals are more susceptible to cryptococcal infections than immunocomprised individuals in China, remain to be further elucidated. Finally, diagnosis and treatment of cryptococcal infections are still a big challenge in many regions (especially the undeveloped western provinces) in China due to limited experience and molecular tools.

Conflict of interest

The authors have no conflict of interests to report.

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