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MEDICINE

First Autochthonous Case of Cryptococcal Meningitis in an Immunocompetent Host Due to *Cryptococcus gattii* VGI in Northern Italy

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Abstract

We describe a case of cryptococcal meningitis diagnosed in an immunocompetent patient, with no apparent predisposing conditions, living in Northern Italy. The patient was initially admitted for visual disturbs with nausea and cervical pain, but magnetic resonance imaging of the brain showed a diencephalic lesion. Colonies of *Cryptococcus gattii* species complex were isolated from cerebrospinal fluid. Antifungal therapy was started and conditions of the patient improved in 2 months. The fungal isolate was identified as *C. gattii* molecular type VGI, mating type αB, and genotype ST156. This is the most common *C. gattii* species complex genotype circulating in Europe and in Italy suggesting that the infection was probably acquired in Italy since the patient did not report any recent travel abroad.

Keywords *Cryptococcus gattii* · Northern Italy · Immunocompetent host · Autochthonous case

Introduction

The basidiomycete yeasts belonging to *Cryptococcus neoformans* and *Cryptococcus gattii* species complexes are the etiological agents of cryptococcosis, a life-threatening fungal disease affecting both immunosuppressed and immunocompetent hosts [1]. Fungal infection is caused by inhalation of basidiospores or small-sized blastospores allowing the pathogen to settle in the lungs from where cryptococcal yeasts can enter the bloodstream and cross blood-brain barrier causing central nervous system infection [2]. Predisposing conditions for host infection include underlying HIV infection, solid organ transplantation, idiopathic $CD4^+$ lymphopenia, corticosteroid or immunosuppressive drug use, chronic lung disease, and other immunosuppressive states such as diabetes, chronic kidney diseases, and autoimmune diseases [3, 4]. Cases of cryptococcosis affecting immunocompetent hosts are also reported. These cases are mostly due to *C. gattii* belonging to molecular type VGI or VGII and they usually manifest as pulmonary disease or cryptococcoma in the lungs or brain [5]. The two species complexes also differ in their geographical distribution. *C. neoformans* species complex occurs worldwide whereas *C. gattii* occurs mainly in tropical and subtropical regions [6] but also in some temperate climate zones such as the Pacific Northwest coast of North America including British Columbia in Canada, and Oregon and Washington states in the USA [7, 8], and Europe and the Mediterranean area [9]. In Italy, cases of cryptococcosis due to *C. gattii* species complex are very rare and only three human cases have been reported in the last 25 years [10–12]. Here, we present the first case of *C. gattii* VGI infection in an immunocompetent host, in Northern Italy.

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Case Report

A 32-year-old woman was admitted to the hospital, in the neurological ward, with a history of nausea, vomit, cervical pain, and diplopia occurring for several days. After eye examination, no lesions or alterations of ocular fundus were observed whereas diplopia was confirmed. By the anamnesis, no previous important pathologies, except kidney lithiasis and esophageal reflux, were reported as well as no drug therapies were recently administered to the patient. Magnetic resonance imaging (MRI) of the brain revealed an enhancement of leptomeninges at the diencephalic left region (Fig. 1a). Cerebrospinal fluid (CSF) collected by lumbar puncture showed evidence of meningitis with a high concentration of lymphocytes (1700/mm^3) and proteins. Cultures of CSF resulted negative for bacteria and virus but supported the growth of white mucous yeast colonies identified as *Cryptococcus gattii* species complex by MALDI-TOF analysis. The patient was then transferred to the infectious diseases ward and treated with liposomal amphotericin B (150 mg/d in three doses) associated with voriconazole (400 mg/d in two doses) since flucytosine was not available. Further laboratory investigations were performed and results confirmed a high titer of *Cryptococcus* antigens (CrAg) in both serum and CSF (1/512 and 1/8, respectively). On the other hand, the patient was negative to HIV, HBV, and HCV antigenic tests, and CD4$^+$/CD8$^+$ cell ratio was normal (1.67). Antifungal susceptibility testing performed on the yeast isolate by the CLSI standard method revealed a high sensitivity to amphotericin B (MIC = 0.5 μg/ml, ECV = 1 μg/ml), itraconazole (MIC = 0.12 μg/ml, ECV = 0.5 μg/ml), voriconazole (MIC = 0.06 μg/ml, ECV = 0.12 μg/ml), posaconazole (MIC = 0.125 μg/ml, ECV = 0.25 μg/ml), and fluconazole (MIC = 4 μg/ml, ECV = 8 μg/ml). After 2 months of therapy, patient conditions constantly improved: cervical pain and diplopia were resolved, CSF cultures were negative, CrAg values in serum decreased to 1/8, and MRI evidenced the reduction of the brain area infected. The patient was then discharged with a consolidation therapy of voriconazole 400 mg administered in two doses per day. Six months later, the patient visited the hospital for a check of her conditions and to evaluate therapy suspension. No neurological disturbs were reported by the patient after discharge. General laboratory analyses were all in the normal ranges. Before therapy suspension, a lumbar puncture for CSF CrAg titration and MRI of the brain were repeated. CSF resulted negative and imaging showed a complete resolution of the pathology (Fig. 1b). Therefore, antifungal therapy was definitely suspended and the patient was discharged.

The *C. gattii* species complex isolate was sent to the Laboratory of Medical Mycology at the Università degli Studi di Milano, Milano, Italy, for further molecular investigations. Molecular type and mating type were determined by multiplex PCR as previously described [13, 14], and multi-locus sequence typing was performed using the standard multi-locus sequence typing (MLST) scheme [15]. The MLST profile was then compared with those present in *C. gattii* MLST database (www.mycologylab.org) in order to find a relationship with other isolates previously identified. The isolate resulted belonging to molecular type VGI and mating type αB whereas its MLST profile was ST156. Comparison with MLST profiles of global isolates (Fig. 2) showed that the *C. gattii* VGI isolate here described was identical to several strains recovered from clinical and

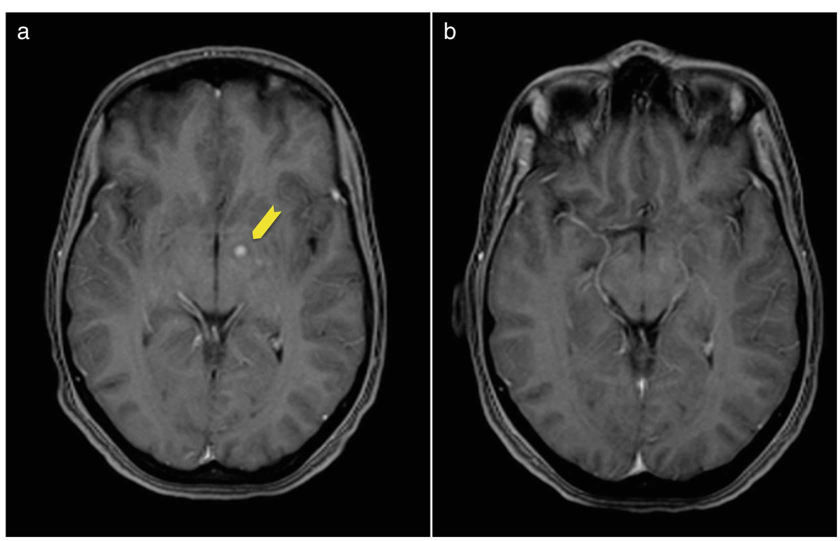

Fig. 1 Magnetic resonance images of the patient brain showing **a** the enhancement of leptomeninges at the diencephalic left region and **b** the complete resolution after 6 months of antifungal therapy

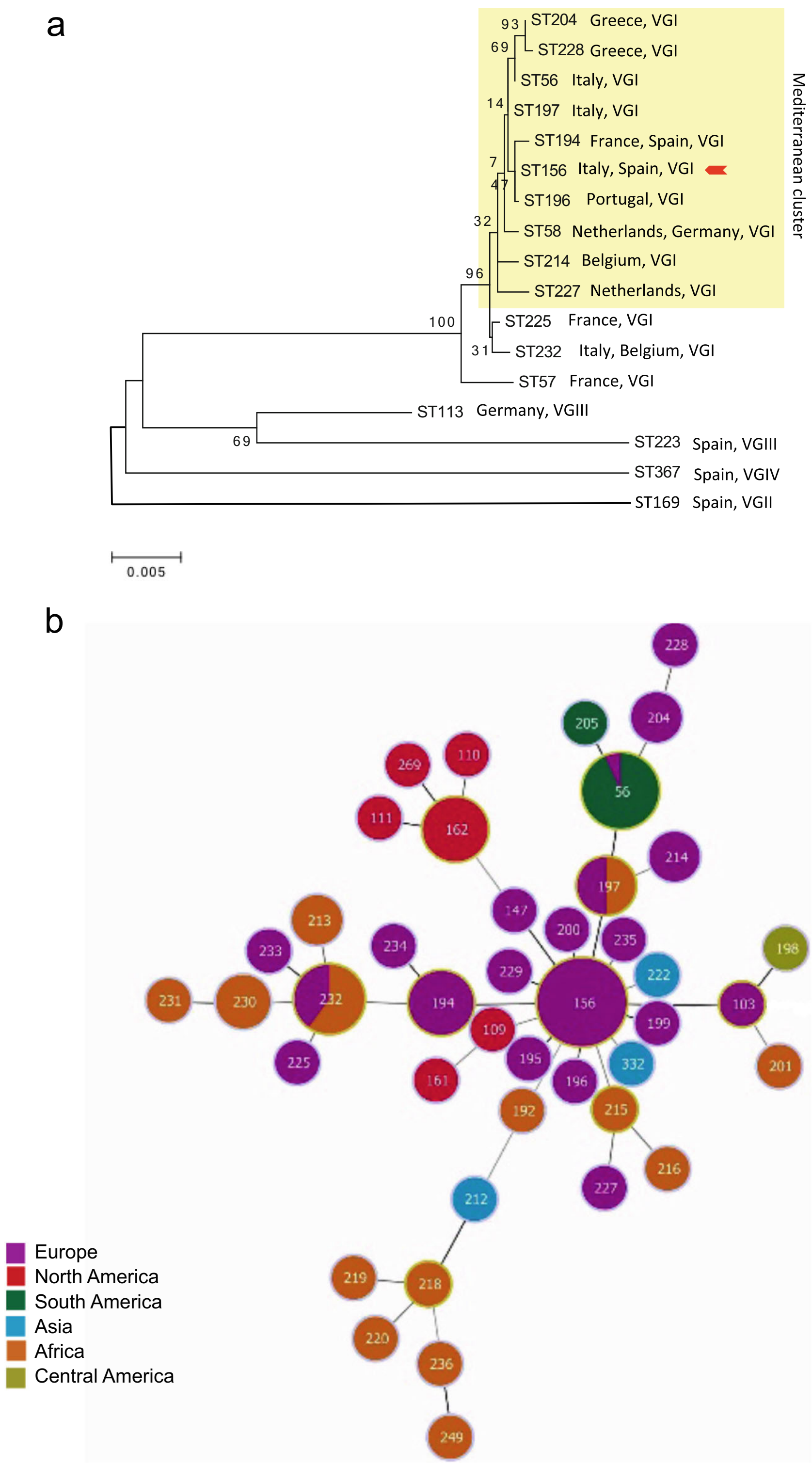

Fig. 2 **a** Maximum likelihood phylogenetic tree showing the main VGI genotypes present in Europe inferred using the concatenated sequences of the seven loci of the standard MLST scheme. Genotypes ST223 (VGIII), ST367 (VGIV), and ST169 (VGII) were used as outgroups. Genotype ST156 is indicated by a red arrow. Genotypes belonging to the Mediterranean cluster are included in the yellow square. Bootstrap analysis values (1000 repeats) are reported at each node. **b** Minimum spanning tree showing the genetic relationship between the main European genotypes and the most related genotypes from other continents. The numbers inside the circles correspond to the genotypes, and the size of each circle is proportional to the number of isolates with the same genotype. Genotypes are linked by thick lines (one locus difference) or thin lines (two loci difference)

environmental sources in southern Italy and Spain [16] and was included in an endemic European cluster.

Discussion

The case here reported is the first case of cryptococcal meningitis in an immunocompetent patient in Northern Italy due to a *Cryptococcus gattii* VGI strain. The patient has no apparently predisposing conditions and no immunosuppressive signs were detected. Since no recent travels abroad in endemic areas were reported by the patient, it is likely that the infection was acquired in Italy. This was confirmed by molecular typing of the fungal isolate which showed it belonged to genotype ST156. This genotype is the most common *C. gattii* VGI genotype present in Europe [16] and in Italy and was previously identified in Apulia region from *Eucalyptus* and olive tree samples, and from a series of samples collected in the cages of exotic animals living in a zoo [17]. The only clinical isolate belonging to this genotype was responsible of disseminated cryptococcosis in an HIV patient living in Bari, in the same Italian region [10] (Table 1). Apulia is one of the most popular seaside locations for summer holidays in southern Italy but it was not investigated if the patient traveled in this region previously had the infection. On the other hand, it cannot be excluded that the infection was acquired in her residence zone, in Northern Italy, since no environmental samples were collected in this area to confirm the presence of the fungus. A previous case of cryptococcosis in an immunocompetent host due to *C. gattii* VGI was again reported from Apulia but the fungal isolate belonged to a different autochthonous genotype (ST232) [12]. Presentation of the above case was different compared with the case here reported. The patient of the former case presented a pulmonary infection with no evident signs of meningitis whereas in our case, the patient had clear signs of meningitis confirmed by MRI of the brain (Table 1). This is probably due to a different stage of the fungal disease observed in the two different cases.

Cryptococcosis in immunocompetent hosts can present unusual clinical manifestations and therefore sometimes, diagnosis is difficult to make in a short time causing complications that can delay the administration of a correct therapy and can be fatal [18, 19]. Although in our case, the patient was considered immunocompetent on the basis of classical immunological tests (immunoglobulin dosage and lymphocyte subpopulation analysis); other immune diseases, not detected by the above methods, cannot be excluded. For example, some authors report that the presence of antibodies anti-granulocyte-macrophage colony-stimulating factor (GM-CSF), not investigated in our case, could be a predisposing condition for *C. gattii* infection [20]. Detection of anti-GM-CSF antibodies should be encouraged in cryptococcosis cases due to *C. gattii* strains in order to elucidate the relationship

Table 1 Cases of cryptococcosis due to *Cryptococcus gattii* reported in Italy including the present case

Case no.	Year of diagnosis	Patient's age and sex	Patient's residence	Immunological status	Predisposing conditions	Initial presentation	Outcome	*C. gattii* strain (molecular type-sequence type)	Reference
1	1992	40, female	Italy and Brazil	Immunocompetent	Travels in endemic areas	Pulmonary infection and meningitis	Survived	VGI-ST56	[11]
2	1995	30, female	Southern Italy	Immunocompromised	HIV infection	Meningitis	Dead	VGI-ST156	[10]
3	2009	37, male	Southern Italy	Immunocompetent	Travels in endemic areas	Pulmonary infection	Survived	VGI-ST232	[12]
4	2016	32, female	Northern Italy	Immunocompetent	Unknown	Meningitis	Survived	VGI-ST156	This study

between the expression of this factor and the progress of the fungal disease. In conclusion, this case report confirms that cryptococcosis can manifest in immunocompetent patients and that clinicians must be aware about this risk when CT scan of the lungs or MRI of the brain shows infectious lesions. This risk should be taken more in consideration in Italy where the presence of *C. gattii* VGI in the environment has been proved with numerous findings and where bioclimatic conditions are optimal for the survival of this pathogen [9].

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

Ethical Approval Ethical approval is not required for this study.

Informed Consent The patient expressed an informed consent for the publication of the present case report.

References

1. Kwon-Chung KJ, Fraser JA, Doering TL, et al. *Cryptococcus neoformans* and *Cryptococcus gattii*, the etiologic agents of cryptococcosis. Cold Spring Harb Perspect Med. 2014;4:a019760.
2. Velagapudi R, Hsueh YP, Geunes-Boyer S, Wright JR, Heitman J. Spores as infectious propagules of *Cryptococcus neoformans*. Infect Immun. 2009;77:4345–55.
3. Rajasingham R, Smith RM, Park BJ, Jarvis JN, Govender NP, Chiller TM, et al. Global burden of disease of HIV-associated cryptococcal meningitis: an updated analysis. Lancet Infect Dis. 2017;17:873–81.
4. Beardsley J, Sorrell TC, Chen SC. Central nervous system cryptococcal infections in non-HIV infected patients. J Fungi (Basel). 2019;5:pii: E71.
5. Chen SC, Meyer W, Sorrell TC. *Cryptococcus gattii* infections. Clin Microbiol Rev. 2014;27:980–1024.
6. Cogliati M. Global molecular epidemiology of *Cryptococcus neoformans* and *Cryptococcus gattii*: an atlas of the molecular types. Scientifica (Cairo). 2013;2013:675213.
7. Engelthaler DM, Hicks ND, Gillece JD, Roe CC, Schupp JM, Driebe EM, et al. *Cryptococcus gattii* in North American Pacific Northwest: whole-population genome analysis provides insights into species evolution and dispersal. MBio. 2014;5:e01464–14.
8. Acheson ES, Galanis E, Bartlett K, Mak S, Klinkenberg B. Searching for clues for eighteen years: deciphering the ecological determinants of *Cryptococcus gattii* on Vancouver Island, British Columbia. Med Mycol. 2018;56:129–44.
9. Cogliati M, D'Amicis R, Zani A, et al. Environmental distribution of *Cryptococcus neoformans* and *C. gattii* around the Mediterranean basin. FEMS Yeast Res. 2016;16:pii: fow086.
10. Montagna MT, Tortorano AM, Fiore L, et al. *Cryptococcus neoformans* var. *gattii* en Italie. Note I. Premier cas autochtone de méningite de sérotype B chez un sujet VIH positif. J Mycol Méd. 1997;7:90–2.
11. Tortorano AM, Viviani MA, Rigoni AL, et al. Prevalence of serotype D in *Cryptococcus neoformans* isolates from HIV positive and HIV negative patients in Italy. Mycoses. 1997;40:297–302.
12. Iatta R, Hagen F, Fico C, Lopatriello N, Boekhout T, Montagna MT. *Cryptococcus gattii* infection in an immunocompetent patient from Southern Italy. Mycopathologia. 2012;174:87–92.
13. Cogliati M, Allaria M, Tortorano AM, Viviani MA. Genotyping *Cryptococcus neoformans* var. *neoformans* with specific primers designed from PCR-fingerprinting bands sequenced using a modified PCR-based strategy. Med Mycol. 2000;38:97–103.
14. Esposto MC, Cogliati M, Tortorano AM, Viviani MA. Determination of *Cryptococcus neoformans* var. *neoformans* mating type by multiplex PCR. Clin Microbiol Infect. 2004;10:1092–4.
15. Meyer W, Aanensen DM, Boekhout T, Cogliati M, Diaz MR, Esposto MC, et al. Consensus multi-locus sequence typing scheme for *Cryptococcus neoformans* and *Cryptococcus gattii*. Med Mycol. 2009;47:561–70.
16. Cogliati M, Desnos-Ollivier M, McCormick-Smith I, et al. Genotypes and population genetics of *Cryptococcus neoformans* and *Cryptococcus gattii* species complexes in Europe and the Mediterranean area. Fungal Genet Biol. 2019;129:16–29.
17. Montagna MT, De Donno A, Caggiano G, et al. Molecular characterization of *Cryptococcus neoformans* and *Cryptococcus gattii* from environmental sources and genetic comparison with clinical isolates in Apulia. Italy Environ Res. 2018;160:347–52.
18. Santander XA, Gutiérrez-González R, Cotúa C, et al. Intraventricular cryptococcoma mimicking a neoplastic lesion in an immunocompetent patient with hydrocephalus: a case report. Surg Neurol Int. 2019;10:115.
19. Khattab A, Patruni S, Sealey ML. Rare presentation of cryptococcal meningitis in an immunocompetent patient. BMJ Case Rep. 2019;12:pii: e230003.
20. Kwon-Chung KJ, Saijo T. Is *Cryptococcus gattii* a primary pathogen? J Fungi (Basel). 2015;1:154–67.