Clinical and microbiological characteristics of *Cryptococcus gattii* isolated from China

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Abstract

Background: Infection, even outbreak, caused by *Cryptococcus gattii* (*C. gattii*) has been reported in Canada and the United States, but there were sparsely-reported cases of *C. gattii* in China. Our interest in occurrence, clinical manifestation, laboratory identification and molecular characterization of Chinese *C. gattii* strains leads us to this research.

Methods: A total of 254 clinical isolates primarily identified as *Cryptococcus neoformans* (*C. neoformans*) were collected. VITEK 2 compact, canavanine glycine bromothymol blue (CGB) agar and Bruker Biotyper matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) were used for strain identification. Multi-locus sequence typing (MLST) was performed for genotyping. Antifungal susceptibility test was carried out with commercial kits of both ATB fungus 3 and Yeast one. Clinical information of patients was reviewed retrospectively. Label-free proteome technique was used to quantitatively analyze the differential proteins of *C. gattii*.

Results: Out of 254 clinical isolates, we identified eight strains as *C. gattii*. MLST showed genotype VGI accounted for the most (6 / 8), the other two strains were genotype VGII，VGIIa and VGIIb respectively，with 3 specific spectra of molecular weight about 4342, 8686, 9611 Dalton by MALDI-TOF MS. The minimal inhibitory concentrations (MICs) of Fluconazole with Yeast one was 2～4 times higher than that with ATB fungus 3. Higher MICs of antifungal agents were exhibited against VGII strains than against VGI strains. *C. gattii* genotype VGII and VGI possessed 418 and 774 specific proteins respectively. Comparative proteome analysis showed that 180 proteins were highly expressed in *C. gattii* VGII and 329 proteins were highly expressed in *C. gattii* VGI. The enrichment of differentially expressed proteins between VGII and VGI was directed to Golgi complex.

Conclusions: Infection by *C. gattii* in China might have been underestimated because of initial mis-identification. Genotype VGI was predominant but VGII was more resistant to antifungal agents. There was significant difference in protein expression profile between VGII and VGI *C. gattii*.

Background

Cryptococcus gattii (C. gattii), one of the most common pathogenic cryptococcal species, primarily infects immunocompetent hosts, in contrast to Cryptococcus neoformans (C. neoformans), which mainly infects immunocompromised individuals[1,2]。Previous studies revealed that C. gattii infection was restricted geographically to tropical and subtropical regions[1 – 3]. However, outbreaks have been recorded in temperate regions, such as British Columbian of Canada and Pacific Northwest of the United States [4,5]. In China, majority of people reside in temperate and subtropical regions with climates suitable for fungal growth and spread, and the incidence of cryptococcal infection increased significantly in recent years,. Unfortunately, little investigation has been carried out on this organism[6]. The following was a retrospective study on epidemiological, clinical and microbiological characteristics of C. gattii isolated in China.

Methods

1. Collection and initial identification of isolates

A total of 254 clinical isolates initially identified as C. neoformans were collected from seven hospitals in China, they were all stored at − 80 °C. All the isolates were sub-cultured onto Sabouraud dextrose agar medium at 25 °C for 48～72 h and identified initially by VITEK 2 Compact (bioMérieux SA, France). Each isolate was also inoculated on canavanine-glycine-bromthymol blue (CGB) agar[7] at 25 °C for 24～120 h, The isolates which showed blue pigmentation on CGB agar were then subjected to further identification.

2. Identification by MALDI-TOF MS

Isolates were re-identified and analyzed by Bruker Biotyper MALDI-TOF MS (Bruker, Daltonik, Bremen, Germany). Briefly, one single colony was smeared directly on the MALDI-TOF MS analysis plate and formic acid was added. After 3～5 minutes, matrix was added and the plate was put into the MALDI-TOF MS instrument with MALDI Biotyper software 3.0 (Bruker Daltonik GmbH) for species identification and dendrogram analysis. Two strains of C.neoformans were included as outgroup control.

3. Analysis by multilocus sequence typing (MLST)

Genomic DNA of each isolate identified as C. gattii was then extracted using the TianGen® TIANamp Yeast DNA Kit (Tiangen Biotech Beijing CO., LTD, China) complying with the manufacturer's instruction, seven genes of unlinked loci were amplified, including six housekeeping genes (CAP59, GPD1, LAC1, PLB1, SOD1, URA5) and one non-coding region gene (IGS1)[8], bi-directional sequencing for each gene was then carried out, sequence comparison for each locus was done according to the method described by ISHAM Cryptococcal Working Group. Sequences were uploaded to the MLST Database for the C.neoformans/C.gattii species complex (http://mlst.mycologylab.org) one by one. A sequence type (ST) number and seven allele type (AT) numbers were given to each isolate. New AT and ST number will be assigned for new sequence. To differentiate VGII subtypes, twelve unlinked genes (SXI1α, TEF1, FTR1, CBP1, ICL1, HOG1, TOR1, STE7, TRR1, FHB1, RAS1, PAK1) were also amplified and sequenced according to the method of Fraser et al[9], they were analyzed and compared with Vancouver Island strains of R265 and R272.

4. Antifungal susceptibility test of C. gattii isolates

Antifungal susceptibility test was carried out by both ATB fungus 3 (bioMérieux SA, France) and Yeast one (Trek Diagnostic Systems Ltd, UK) following their instructions. Candida krusei ATCC 6258 and Candida parapsilosis ATCC 22019 were used for quality control.

5. Clinical information of patients

Under authorization of Medical Record Department, Clinical information of patients infected by C. gattii was reviewed retrospectively, including age, sex, underlying diseases, symptoms, imaging findings, laboratory examinations and antifungal therapy.

6. Analysis of differential proteins

According to the regional distribution and the integrity of clinical data of the strains, six strains (4 of VGI and 2 of VGII) of C. gattii were selected for proteome analysis. The proteins were first extracted from the strains, after being separated by SDS-PAGE electrophoresis, the proteins were hydrolyzed and analyzed. Original mass spectrum files of 6 strains were imported into Maxquatt software (version 1.3.0.5) for analysis. The database source was for C.gattii. in Uniprot(http://www.geneontology.org/). Plus or minus 15 ppm of polypeptide molecular weight, or missing 2 cutting sites were set as Maxquatt parameters, false discovery rate (FDR) for polypeptide identification was set as 0.01. A standard of $p < 0.05$ and 2-fold expression were considered as different proteins. The enrichment and pathway analysis was carried out by using the GO plot package of R language and KEGG online tool (https://david.ncifcrf.gov/, http://www.kegg.jp/kegg/pathway.html). The bubble diagram was drawn according to the R language ggplot2 package.

Results

1. Identification and distribution of C. gattii isolates

Among 254 collected Cryptococcal strains, eight showed blue pigmentation on CGB agar and were characterized as C.gattii by MALDI-TOF MS with scores from 2.043 to 2.275 although they were all identified as C.neoformans by VITEK 2 compact. All these eight isolates were positive for urease test as seen in Table 1.

Table 1
Identification of 8 C. gattii strains by different methods.

Isolate No.	ID by VITEK 2 Compact (Identification rate)	pigmentation on CGB agar	ID by MALDI-TOF MS (score)	Urease test
1	C. neoformans (93%)	blue	C. gattii (2.190)	positive
2	C. neoformans (90%)	blue	C. gattii (2.043)	positive
3	C. neoformans (96%)	blue	C. gattii(2.275)	positive
4	C. neoformans (99%)	blue	C. gattii (2.092)	positive
5	C. neoformans (86%)	blue	C. gattii (2.118)	positive
6	C. neoformans (91%)	blue	C. gattii (2.126)	positive
7	C. neoformans (98%)	blue	C. gattii (2.188)	positive
8	C. neoformans (99%)	blue	C. gattii (2.194)	positive

The eight strains of C. gattii were isolated from eight patients. Two patients with isolate numbers of 2 and 6 were from Hainan and Yunnan Province, which were recognized as tropical areas. Three patients with isolate number 3 (from Chongqing city), 4 and 7 (from Fujian Province) were recognized as being from subtropical area. The other three patients with isolate numbers of 1, 5 and 8 were from Shandong Province, Inner Mongolia Autonomous Region and Henan Province, which were recognized as temperate regions.

2. Results of multilocus sequence typing (MLST)

Six strains of C. gattii possessed genotype VGI and two strains possessed genotype VGII as indicated in Table 2. Through further analysis and comparison with Vancouver Island reference stains of R265 and R272, the 2 strains of isolate number 1 and 6 with C.gattii genotype VGII could be subtyped into VGIIa and VGIIb respectively as in Table 3. Some gene mutation existed in unlinked gene loci such as FTR1 and RAS1 as indicated in Fig. 1.

Table 2
MLST profiles and Genotype of 8 C. gattii strains.

isolate No.	Multilocus sequence typing profile (Allele number)							STs	Genotype
	CAP59	GPD1	IGS1	LAC1	PLB1	SOD1	URA5		
1	1	1	4	4	1	14	7	20	VGII
2	36	11	13	5	13	36	14	106	VGI
3	16	5	3	5	5	65	12	57	VGI
4	16	11	46	13	13	34	15	197	VGI
5	16	5	3	5	5	65	12	57	VGI
6	2	6	10	4	2	15	2	7	VGII
7	16	5	3	5	5	65	12	57	VGI
8	16	33	44	13	13	47	24	161	VGI

Table 3
MLST profiles of 16 gene loci for 2 C. gattii strains with genotype VGII Notes: R265 and R272 were Vancouver Island reference strains for C. gattii genotype VGIIa and VGIIb respectively; / represented that data was not available; GenBank accession numbers for multilocus sequence typing alleles were: CAP59-1, DQ096432; CAP59-2, DQ096433; GPD1-1, DQ096377; GPD1-6, DQ096382; IGS-4, DQ096314; IGS-10, DQ096319; PLB1-1, DQ096343; PLB1-2, DQ096344; SXI1α-18, DQ096308; SXI1α-19, AY973646; TEF1-7, DQ096364; TEF1-5, DQ096362; FTR1-1, DQ096448; FTR1-2, DQ096449; CBP1-1, DQ096435; CBP1-2, DQ096436; ICL1-1, DQ096458; ICL1-2, DQ096459; HOG1-1, DQ096456; TOR1-1, DQ096470; STE7-1, DQ096467; STE7-2, DQ096468;TRR1-1, DQ096472; TRR1-2, DQ096473; RAS1-1, DQ096464; RAS1-2, DQ096465; PAK1-1, DQ096461; PAK1-2, DQ096462.

isolate NO.	Multilocus sequence typing profile (Allele number)															
	CAP59	GPD1	IGS1	PLB1	SXI1α	TEF1	FTR1	CBP1	ICL1	HOG1	TOR1	STE7	TRR1	FHB1	RAS1	PAK1
R265	1	1	4	1	18	7	1	1	1	1	1	1	1	1	1	1
1	1	1	4	1	/	7	/	1	1	1	1	2	1	1	/	1
R272	2	6	10	2	19	5	2	2	2	1	1	2	2	1	2	2
6	2	6	10	2	/	/	/	2	2	1	1	2	2	1	/	/

3. Mass spectra of C. gattii isolates

As shown in Fig. 2, the mass spectra obtained from eight C. gattii isolates were characterized by diverse spectra in the range between 2000 and 10000 Da (Dalton). Three specific spectra with molecular weight of about 4342, 8686, 9611 Dalton could be seen from two isolates of C. gattii VGII, which were absent in six isolates of C. gattii VGI. The dendrogram by MALDI Biotyper could divide eight isolates of C. gattii into C. gattii VGI and VGII group as shown in Fig. 3.

4. Results of antifungal susceptibility test

The MICs of six antifungal drugs to eight strains of C. gattii were listed in Table 4.

Table 4

Results of antifungal susceptibility test by ATB fungus 3 and Yeast one Notes: a and b represented the method of ATB fungus 3 and Yeast one respectively; the abbreviations for antifungal drugs were: 5-FU: 5-Flucytosine; AMB: Amphotericin B; FCA: Fluconazole; ITR: Itraconazole; VRC: Voriconazole; PSZ: Posaconazole.

isolates	5-FU		AMB		FCA		ITR		VRC		PSZ
	a	b	a	b	a	b	a	b	a	b	b
1	< 4	2	< 0.5	1	8	32	0.25	0.25	0.25	0.25	0.25
2	< 4	0.5	< 0.5	0.25	1	4	< 0.125	0.06	0.06	0.06	0.06
3	< 4	0.5	< 0.5	0.25	1	4	< 0.125	0.12	0.06	0.12	0.12
4	< 4	1	< 0.5	0.25	2	4	< 0.125	0.06	< 0.125	0.06	0.12
5	< 4	0.5	< 0.5	0.25	2	4	< 0.125	0.12	0.06	0.06	0.12
6	< 4	2	< 0.5	0.5	8	128	0.25	0.25	0.25	0.25	0.5
7	< 4	0.5	< 0.5	0.25	1	4	< 0.125	0.06	0.06	0.06	0.12
8	< 4	1	< 0.5	0.25	2	4	< 0.125	0.12	0.06	0.06	0.12

5. Clinical information of patients infected with C. gattii

Information of patient infected with isolate number 3 was unavailable, the other 7 patients were immunocompetent young male adults, with age from 21 to 60 years old. Their clinical and laboratory information were summarized in Table 5.

Table 5
Clinical and laboratory information for patients infected with C. gattii Notes:+ and – represented symptoms appeared and not appeared; NA represented data not available.

	1	2 4	5	6		7	8
Location (Province)	Shangdong	Hainan	Fujian	Neimeng	Yunnan	Fujian	Henan
History(medical/contact)	No	No	No	No	No	No	No
Immunity	Normal	Normal	Normal	Normal	Normal	Normal	Normal
Fever	+	+	+	-	+	+	+
Headache	+	+	+	+	+	+	-
Nausea and Vomiting	-	+	+	+	-	-	-
Seizure	-	-	+	-	-	-	-
Neck stiffness	+	-	+	+	-	-	-
Kernig's sign	+	+	+	-	-	-	-
Papilloedema	-	-	+	+	-	-	-
Lung CT	Irregular nodule with spicules and lobulation	Left lower lung mass	Normal	Left lung mass	Left lower lung consolidation	Multiple small nodules	Right upper lung mass
Brain MRI	Meningeal lesions	Normal	Meningeal enhancement	Left lacunar infarction	NA	Normal	Normal
Organism also found by lung biopsy	Yes	NA	NA	NA	NA	Yes	Yes
Blood culture	No growth	No growth	No growth	NA	NA	NA	No growth
Cryptococcal antigen titre in serum	1:1024	NA	NA	NA	NA	NA	1:640
CSF test							
Pressure (mm H2O)	260	330	330	330	NA	200	normal
Glucose (mmol/L)	0.5	4.11	2.49	2.3	NA	2.22	normal
Protein (g/L)	1.57	0.71	0.35	0.73	NA	29	normal
Chloride (mmol/L)	111	144.7	117	102	NA	126.6	normal
White blood cells(× 10^6 cells/L)	377	80	720	91	NA	368	normal
Ink staining	positive	positive	positive	positive	NA	NA	positive
Cryptococcal antigen titre	1:1024	NA	NA	NA	NA	NA	1:640
Organism cultured from/on	CSF/NA	CSF/2015.12	CSF/NA	CSF/ 2016.6.8	sputum/2016.7.26	CSF/ 2015.9.6	sputum/ 2016.8.22
Antifungal therapy	Flu + AmB	Flu + AmB	Flu + AmB	Flu + AmB	Flu + AmB	Flu + AmB	Flu + AmB
Improvement (follow-up)	Obvious	Obvious	Slight	Obvious	Slight	partly	Obvious
Neurological sequelae	No	No	Yes	No	No	No	No

6. Differential protein analysis between C. gattii VGII and VGI

Numbers of identified peptides and proteins were showed in Table 6. A total of 3628 proteins were identified, of which, 2436 proteins were found in both VGII and VGI C. gattii, however, C. gattii genotype VGII and VGI possessed 418 and 774 specific proteins respectively. Comparative proteome analysis showed that 180 proteins were highly expressed in C. gattii VGII and 329 proteins were highly expressed in C. gattii VGI as shown in Fig. 4. A cluster analysis of the differentially expressed proteins was shown in Fig. 5, which clearly demonstrated that the protein expression profile of VGII C.gattii was significantly different from that of VGI C.gattii.

Table 6
Number of peptide and protein in 6 strains of C. gattii

isolates	genetype	Protein	Peptide
1	VGII	1525	6890
6	VGII	2364	12100
2	VGI	2128	11303
5	VGI	2412	12391
4	VGI	1579	6690
8	VGI	2212	12031

The information of GO term and KEGG metabolic pathway analysis exhibited that the enrichment of differential expressed proteins between VGII and VGI was mainly directed to Golgi apparatus, Golgi membrane and Golgi vesicle (as shown in Table 7). The most significant enriched metabolic pathways were oxidative phosphorylation, ribosome and metabolic pathway (as shown in Fig. 6).

Table 7
Results of GO enrichment analysis for Cellular Component

GO ID	GO Term	GeneRatio	BgRatio	Pvalue
GO:0005794	Golgi apparatus	40	51	3.26E-07
GO:0000139	Golgi membrane	26	32	1.37E-05
GO:0005798	Golgi-associated vesicle	12	16	0.011314374
GO:0031982	vesicle	17	26	0.021147847
GO:0030662	coated vesicle membrane	14	21	0.028770779
GO:0044433	cytoplasmic vesicle part	15	23	0.030922717
GO:0034708	methyltransferase complex	4	4	0.036668632
GO:0030120	vesicle coat	12	18	0.042486706
GO:0098796	membrane protein complex	50	95	0.046997979
GO:0030135	coated vesicle	14	22	0.047857677
GO:0031988	membrane-bounded vesicle	15	24	0.049777137

Discussion

1. Selection of identification method for C. gattii

C. neoformans and C. gattii are two important fungal pathogens for humans and animals. Both of them are round with capsule and urease test positive. These routine laboratory identification methods are unable to distinguish the two species[10]. In this study, we analyzed 254 strains primarily identified as C. neoformans, 8 (3.1%) showed pigmentation on CGB agar and were then identified as C. gattii by Bruker MALDI-TOF MS with software 3.0. However, VITEK 2 compact could not differ C.gattii from C. neoformans. Although there were several reports about infection of C. gattii from China[11 – 13], the number of C. gattii infections may have been underestimated because most laboratories utilize biochemical methods for routine identification of cryptococcal species (such as VITEK 2 compact). There might be more cryptococcal cases of C. gattii if appropriate identification methods had been used.

CGB agar was recommended to identify C. gattii from C. neoformans due to its convenience and low cost[10], but it took longer time to get the result. Molecular method would be faster and more accurate for differentiation of the two pathogenic Cryptococci. In this study, eight strains of C. gattii were correctly and timely identified by Bruker MALDI-TOF MS with software 3.0. Additionally, our study showed that C. gattii VGII possessed three specific spectra with molecular weight of about 4342, 8686, 9611 Dalton compared with C. gattii VGI, therefore, we suggest that Bruker MALDI-TOF MS with software 3.0 should be used not only to identify C. gattii but also to differentiate C. gattii VGII from C. gattii VGI. Of course, more research should be done to observe the spectrum difference between C. gattii VGII and other genotypes of C. gattii.

2. Molecular and epidemical characteristics of Chinese C. gattii strains

Four genotypes of C. gattii have been recognized up to now, they were VGI, VGII, VGIII and VGIV. Lockhart and colleagues had reported that VGII and VGIII were the most-frequently identified isolates in America, VGIV were almost exclusively in Africa, and VGI predominated in Europe, Australia and Asia[14]. Our study indicated six strains of C. gattii as genotype VGI (predominant genotype) and two strains as genotype VGII(VGIIa and VGIIb respectively). C. gattii,

as an important primary pathogen, caused outbreak in British Columbia, Canada and the Pacific Northwest, the United States. But 8 strains in our study were pathogens causing sporadic infections according to the strain origination and the isolation time.

3. Drug resistance of Chinese C. gattii strains

The susceptibilities of C. gattii strains to conventional and new antifungal compounds were of significance[15]. Our study investigated susceptibility of eight C. gattii to eight kinds of antifungal agents with two kinds of commercial kits. It was remarkable that the results were in good agreement by two kits, except that MICs of Fluconazole were 2–4 times higher by Yeast one than by ATB fungus 3. All the C.gattii isolates were susceptible to 5-Flucytosine, Amphotericin B, Itraconazole, Voriconazole and Posaconazole. While only one strain was resistant to Fluconazole with MIC of 128 by Yeast one. However, interpretation of MICs of antifungal agents including Fluconazole for category of “susceptibility” or “resistance” was hampered by the lack of clinical breakpoints for C. gattii. Some previous studies have shown that C. gattii may be less susceptible to antifungal agents compared with C. neoformans. In a study from Taiwan, C. gattii was less susceptible to 5Flucytosine and amphotericin B, and in Spain, MICs of Fluconazole, Voriconazole and Posaconazole to C. gattii were significantly higher[16,17].Our data indicated that antifungal agents exhibited higher MICs against isolates of genotype VGII than genotype VGI, which agreed with the data of Hagen et al and Lockhart et al.[18–20,21]. The correlation between susceptibility profile and genotype of C. gattii has rarely been studied[18], however, Clinical relevance between MIC breakpoints and epidemiological MIC cut-off values (ECVs) based on MIC distributions of wild-type strains has currently been studied on both C. neoformans and C. gattii isolates from Europe, USA, Australia, Brazil, Canada, India and South Africa. ECVs of Amphotericin B (0.5–1 μg/ml), 5-Flucytosine (4–16 μg/ml), Fluconazole (8–32 μg/ml) and other azoles varied similarly by molecular type for both C. neoformans and C. gattii [19,20].

4. Clinical manifestations of patients infected with C. gattii

All the patients in our study had no recent travel history overseas, the C. gattii infection occurred domestically. They were immunocompetent young male adults with age from 21–60 years old, which was considered as the reason for increased exposure to environmental sources and increased chance for infection [22]. Most patients had headache and fever, three patients showed neck stiffness, positive Kernig’s sign and meningeal lesions or lacunar infarction by brain MRI. However, most patients (except one only with meningeal enhancement) demonstrated irregular nodule, spicules, lobulation, consolidation and mass by lung CT respectively, which was consistent with the research made by Ngamskulrungroj who indicated even though C.gattii failed to cross the blood-brain barrier and cause fatal meningoencephalitis, C. gattii could cause fatal lung infection [23].

It was reported that host response varied based on the genotype of the organism and concomitant illnesses[24]. There was also study which revealed that VGIIa isolates were more virulent than VGIIb isolates independent of their clinical or environmental origin[25]. In our study, all patients were treated by Fluconazole plus amphotericin B, most of the patients improved effectively without severe neurological sequelae, this might be explained by the fact that Chinese patients infected by C. gattii VGI more than C. gattii VGII on the one hand, and on the other hand, two patients were infected with C. gattii VGII (VGIIa and VGIIb respectively) which showed sequence mutations that might decrease the virulence.

5. Analysis of differentially expressed protein from two genotypes of C. gattii

In this study, the proteome expression profiles of two strains of VGII and four strains of VGI C. gattii were created. A total of 3628 proteins were identified, of which 774 were VGII specific proteins, 418 were VGI specific proteins, and 2436 were common proteins for both VGI and VGII. 180 VGII high-profile proteins and 329 VGI high-profile proteins were obtained by differential protein screening, further cluster analysis showed significant difference in protein expression spectra between VGII and VGI.

GO is a very important method and tool in the field of biological information. By establishing a set of controlled words with dynamic form, the attributes of genes and gene products in organisms can be described comprehensively. KEGG is the main public database with which the most important biochemical metabolic pathways and signal transduction pathways can be determined. Our results suggested that the differential protein of C. gattii VGII and VGI was mainly located on the organelles associated with the Golgi body, that meant energy metabolism of C. gattii might be involved in the difference of pathogenesis mechanism for different genotype of C. gattii. So it was suggested that study on the secretory protein and the secretory vesicles of the two types of C. gattii would be beneficial to the understanding of the pathogenesis of C. gattii, which provided another cutting angle for further study.

Conclusions

In a word, there was not enough C. gattii isolates, the results of our study was within limitation, but our results demonstrated that infection by C. gattii in China might have been underestimated because of initial mis-identification. Genotype VGI was predominant but VGII was more resistant to antifungal agents. C. gattii VGII isolates possessed obvious protein peaks with molecular weight of approximate 4342, 8686, 9611 Dalton. The full spectrum data of C.gattii were provided for the first time, a total of 3628 proteins were identified, 418 and 774 specific proteins were found from C. gattii genotype VGII and VGI respectively. The enrichment of differentially expressed proteins between VGII and VGI was mainly directed to Golgi complex. It will lay a foundation for better understanding and further research on the pathogenesis mechanism for different genotype of C. gattii.

Abbreviations

AT allele type

CGB canavanine glycine bromothymol blue

C. gattii Cryptococcus gattii

C. neoformans Cryptococcus neoformans

FDR false discovery rate

MALDI-TOF MS matrix-assisted laser desorption/ionization time-of-flight mass spectrometry

MICs minimal inhibitory concentrations

MLST multi-locus sequence typing

ST sequence type

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: All authors agreed for publication.

Availability of data and materials: The datasets used and/or analysed during the current study are available from the corresponding author and first author on reasonable request.

Competing interests: The authors declare that there are no competing interests.

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Authors' contributions：DXS designed and organized this study, revised the manuscript, LJ performed most of the experiments and wrote the manuscript； XYX collected strains, analyzed the sequence, HW, LFW and LG collected and identified strains, JRC performed and analyzed MALDI-TOF MS data. All authors read and approved the final manuscript.

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Figures

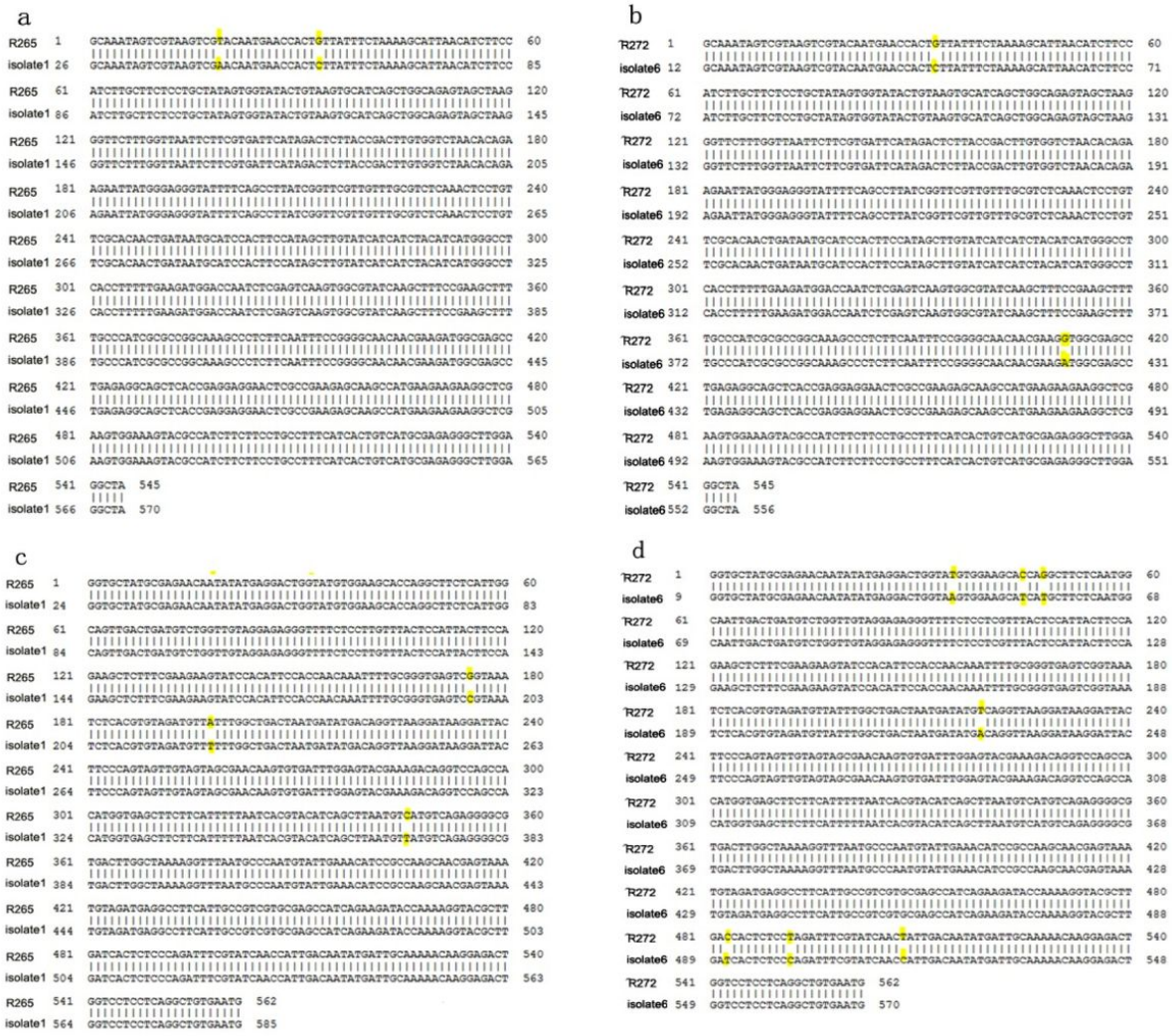

Figure 1

Gene mutations of 2 VGII strains in unlinked gene loci FTR1 and RAS1 compared with reference strains. Notes: a and c demonstrated differential gene loci FTR1 and RAS1 respectively between isolate number 1 and R265, b and d demonstrated differential gene loci FTR1 and RAS1 respectively between isolate number 6 and R272.

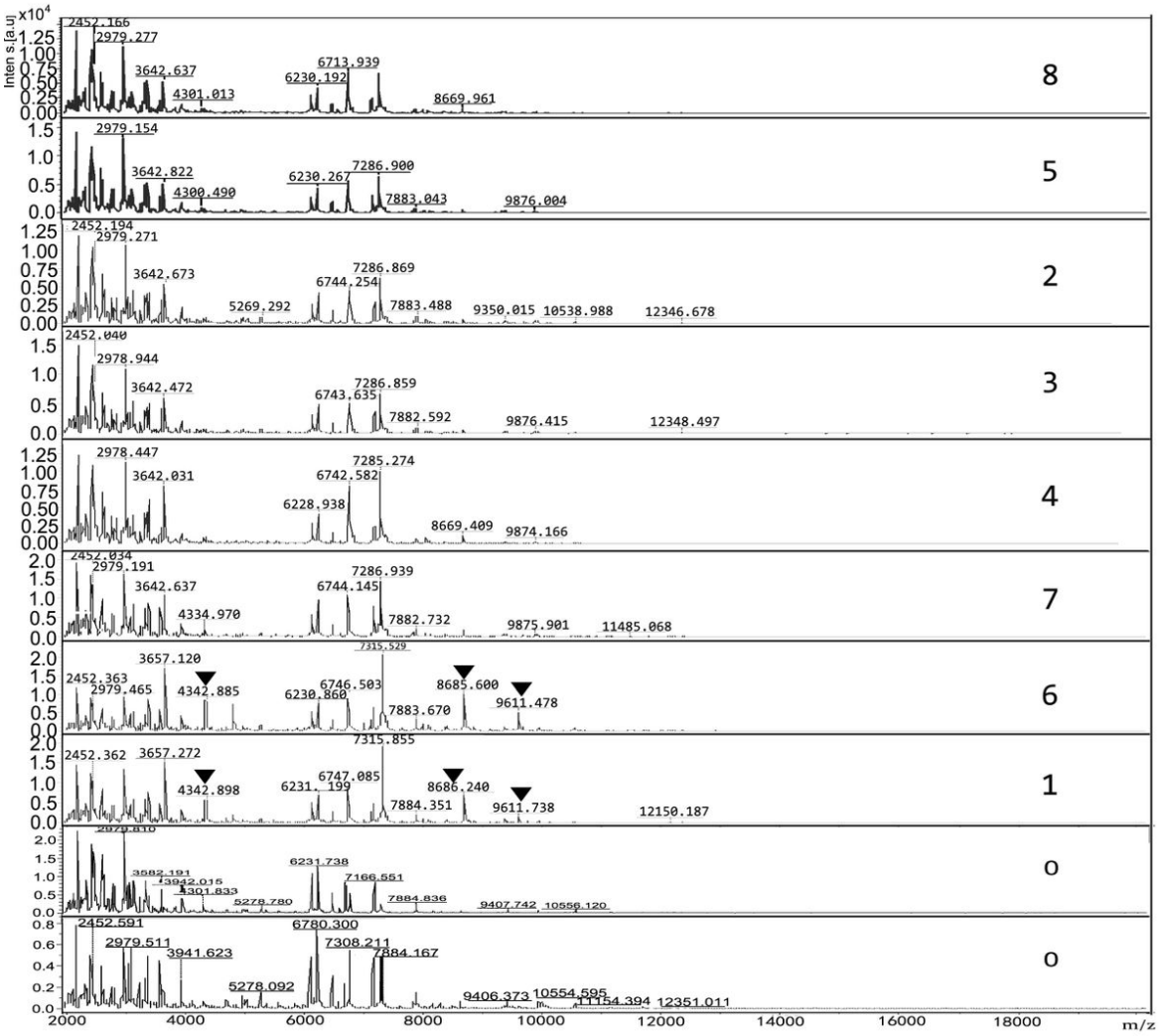

Figure 2

Mass spectra of 8 strains of C. gattii and 2 strains of C.neoformans by MALDI-TOF MS. Notes: Three specific spectra of C. gattii VGII with molecular weight of about 4342, 8686, 9611 dalton were indicated as arrows; 0 represented C.neoformans.

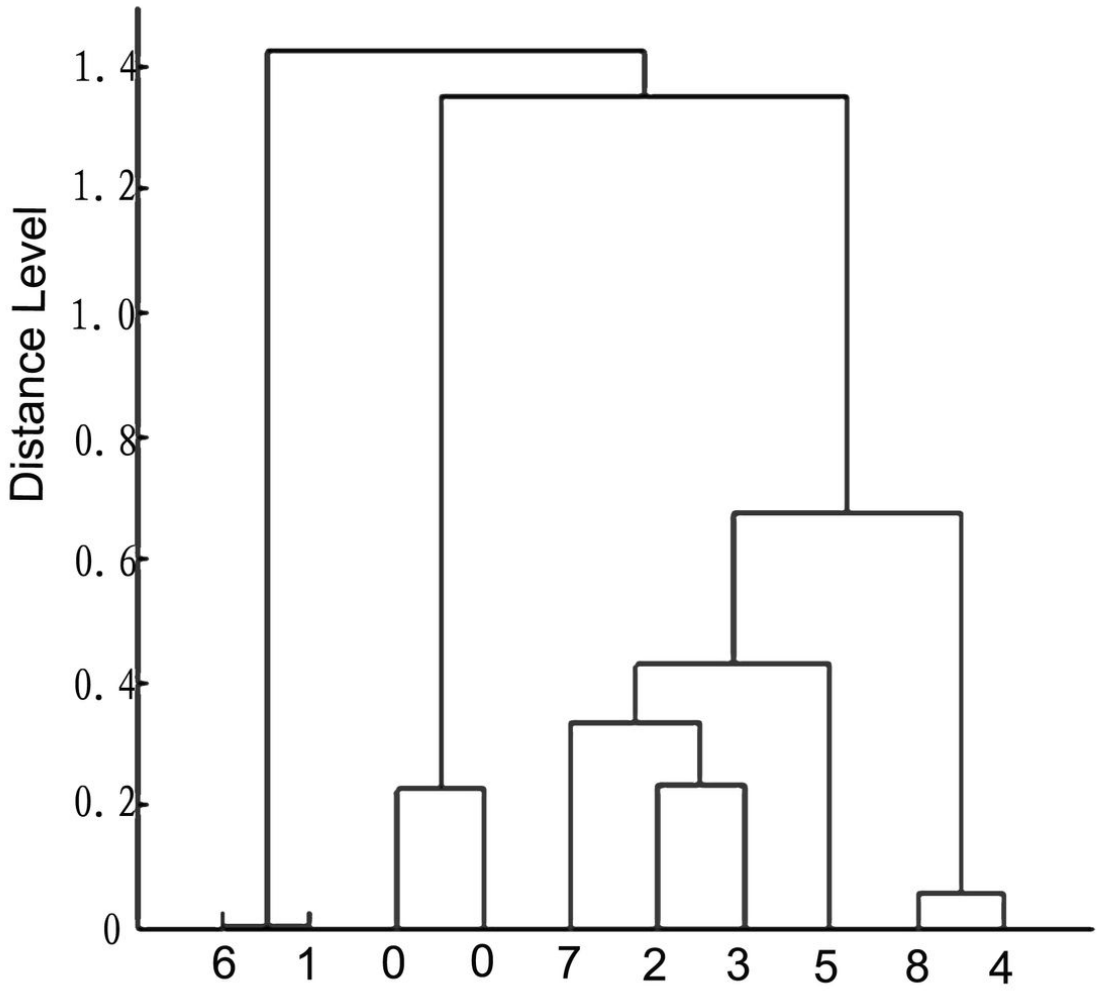

Figure 3

The dendrogram of 8strains of C. gattii and 2 strains of C.neoformans. Notes: 0 represented C.neoformans.

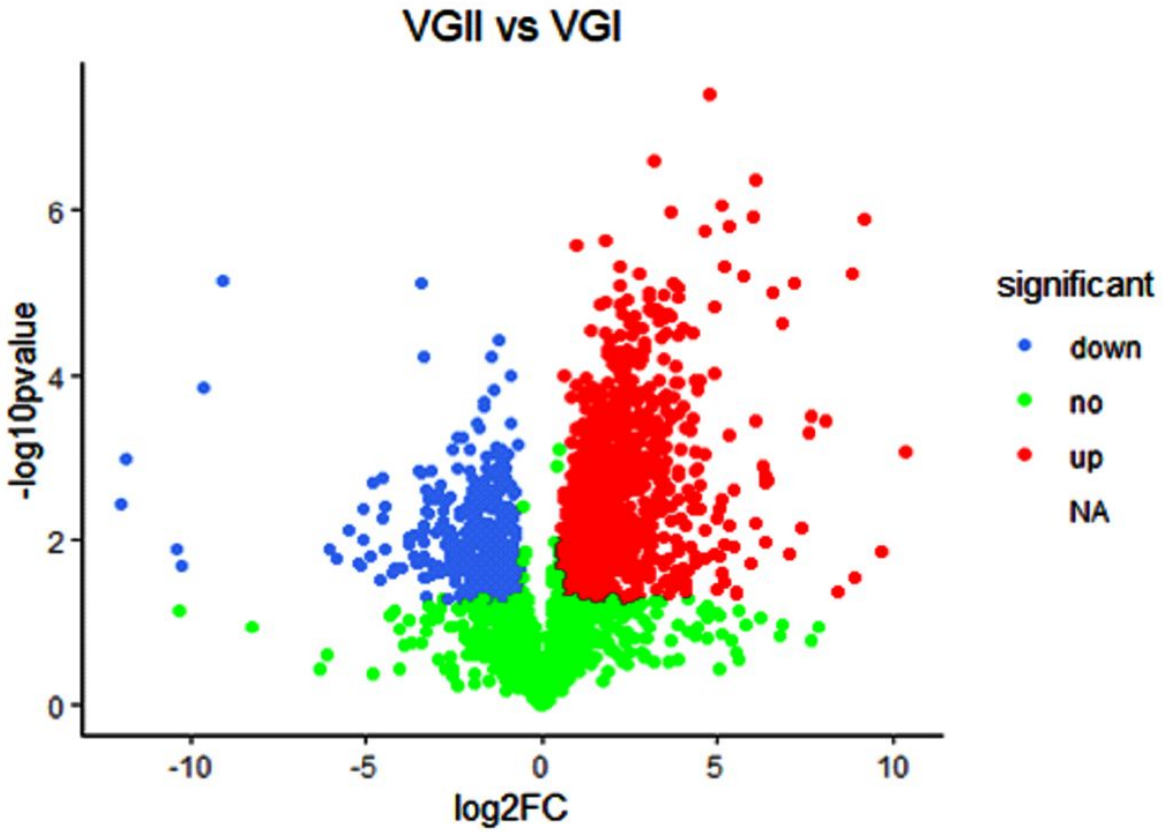

Figure 4

Volcanic map of identified proteins. Notes: The red dot represented the high profile for 329 proteins of C.gattii VGI ($p < 0.05$), the blue dot represented high profile for 180 proteins of C.gattii VGII, and the green dot represented the proteins without significant difference in expression abundance.

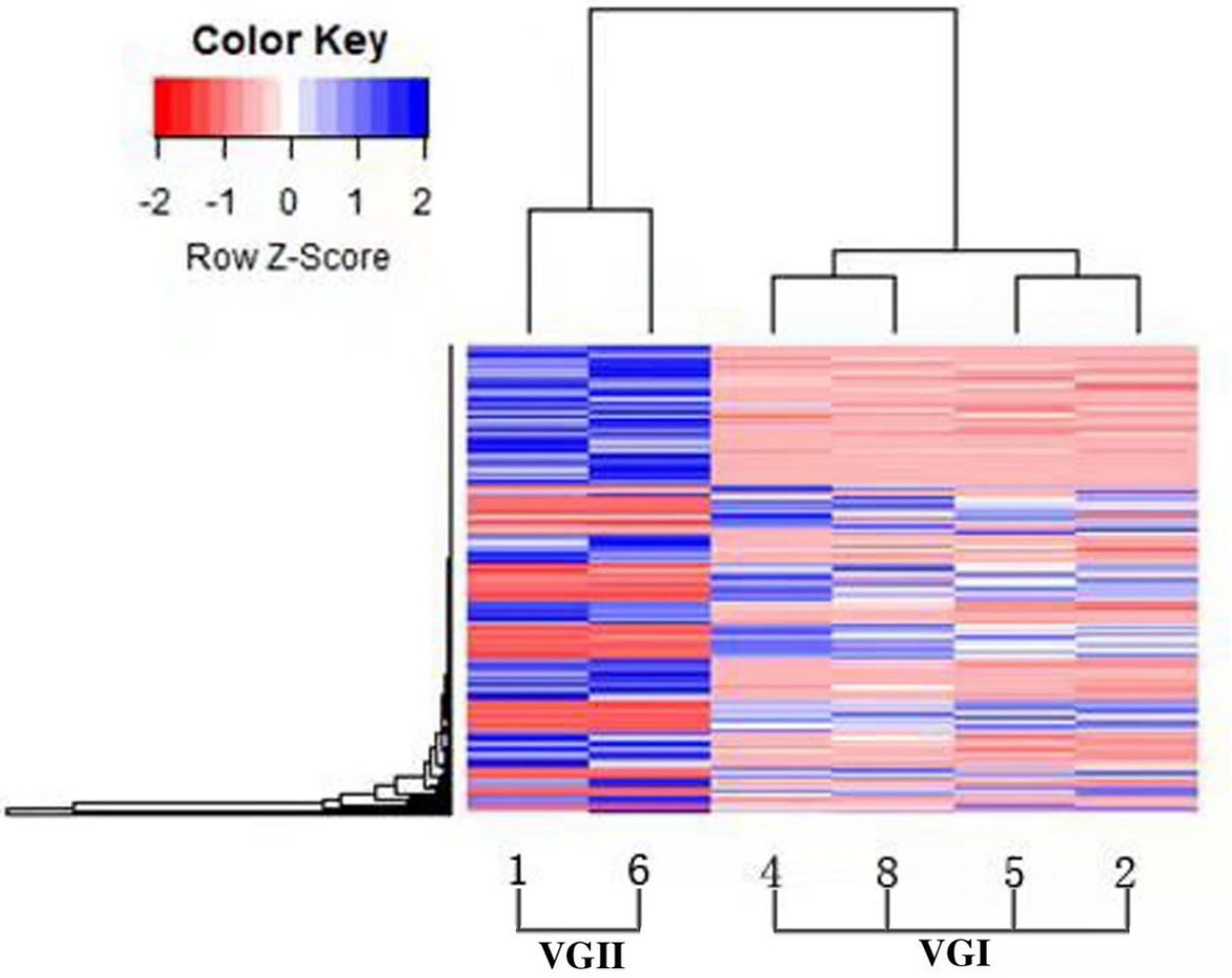

Figure 5

Clustering of differential expression proteins. Notes: Blue and red represented high and low expression proteins respectively.

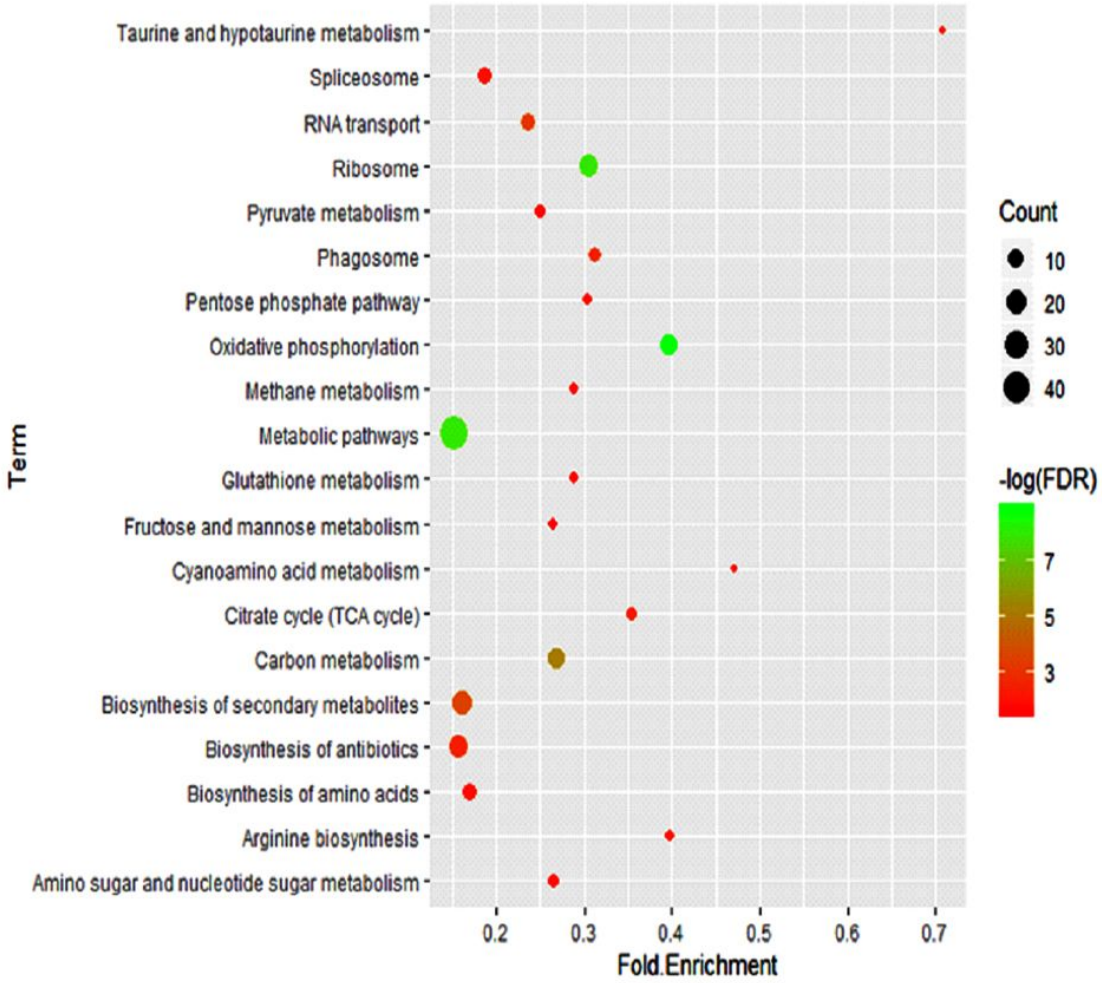

Figure 6

Enrichment Analysis of differential proteins by KEGG metabolic pathway. Notes: The circle was used to represent the enrichment of each GO term, the diameter of the circle represented the number of differentially expressed genes commented to each GO term species, the color of the circle represented the significance of the corresponding GO term (p value corrected by FDR), the redder the color, the more significant the GO term. Bottom transverse coordinates represented “Rich factor”, the larger the value, the higher the proportion of the number of differentially expressed genes to the total number of corresponding GO term genes, that is to say, the richer the number of differentially expressed genes.