(R-WTM) was significantly higher ($P < .05$) than those with mutation (R-WM) and the sensitive isolates (3.2-11 vs. 0.2-2.5, and 0.3-2.2 folds, respectively). Although the R-WTM and R-WM had higher ($P < .05$) CDR2 and MRR1 expression compared to S isolates, noticeable variation was not seen among the other genes. Protein homology modeling and molecular docking revealed that the mutations in the ERG11 gene were responsible for structural alteration and low binding efficiency between ERG11p and ligands. Isolates with ERG11 mutations also presented A220C in ERG1 and together T503C, G751A mutations in UPC2.

Conclusions: Nonsynonymous mutations in the ERG11 gene and coordinated overexpression of various genes including different transporters, ergosterol biosynthesis pathway, transcription factors, and stress-responsive genes are associated with azole resistance in clinical isolates of *C. tropicalis*.

**S5.3a**
**Unraveling the genetic determinants of virulence in *Cryptococcus neoformans***

Katrina Jackson[1], Diana Kisakye[2], Marissa Macchietto[1], Peter Tiffen[1], Kirsten Nielsen[1]
[1]University of Minnesota, Minneapolis, US
[2]Makerere University, Kampala, Uganda

S5.3 Cellular pleomorphism and fungal virulence, September 22, 2022, 3:00 PM - 4:30 PM

*Cryptococcus neoformans* is a human pathogenic basidiomycete yeast that can cause cryptococcal meningitis (CM), predominantly in immunocompromised individuals. The patient outcome depends on both host and pathogen-specific factors, including *C. neoformans* genetics. A groundbreaking 2012 study was the first to show that patient outcome is associated with genetic differences between *C. neoformans* isolates. Subsequent population-wide sequencing studies have revealed over 100 sequence types (ST) of *C. neoformans* that are associated with both geographic location and clinical outcome. All these studies have been broad, examining the severity of disease cryptococcal phenotypes in a collection of highly diverse strains. We chose a narrow focus and collected various genotypic and phenotypic data from a single ST: ST93. ST93 is a common sequence type isolated from patients globally and is the most common clinical isolate found in the sub-Saharan African country of Uganda. Previously, we performed whole genome sequencing on 38 ST93 Ugandan clinical isolates. We identified 652 unique SNPs in this ST93 population compared to the H99 reference genome. We also showed that ST93 contained two subpopulations: ST93A and ST93B. In the current study, we further characterized the genotypic, phenotypic, and virulence differences between these 38 clinical isolates. Using Illumina sequence data, we identified a pattern of linkage disequilibrium that suggested that ST93A and ST93B are evolving separately. We performed long-read sequencing on each isolate to investigate chromosomal changes and large structural variations, allowing us to identify a chromosomal translocation event wherein parts of chromosome 11 had recombined with chromosome 3. Additionally, we characterized several in vitro phenotypes for each isolate and identified three distinct phenotypic clusters based on cell wall challenge and growth experiments. Next, we infected mice with 35 isolates and observed eight different disease manifestations, including isolates that caused non-CNS infections. Overall, by working within a single sequence type, we can gain a deeper understanding of how some small genetic changes can impact strain-specific phenotypes while others have no discernable effect. Eventually, these data can be used to provide valuable information about how each clinical isolate impacts patient outcomes.

**S5.3b**
**Fungal spores: Initiators of colonization and infection**

Tim Baltussen[1], Tom van den Brule[2], Jan Dijksterhuis[2]
[1]Radboudumc, Nijmegen, Netherlands
[2]Westerdijk Fungal Biodiversity Institute, Nijmegen, Netherlands

S5.3 Cellular pleomorphism and fungal virulence, September 22, 2022, 3:00 PM - 4:30 PM

Fungi produce asexual and sexual spores for reproduction and distribution, which can be both in space and time. Distribution in space occurs, by air movement, but also, by water or other vectors such as living organisms. Filamentous fungi from the division Ascomycota that belong to the order Eurotiales produce asexual spores called conidia. Conidia are moderately stress-tolerant cells and are able to survive unfavorable conditions such as thermal stress, dehydration, osmotic pressure, oxidative stress, variations in pH, and UV. For example, conidia of the fungus *Penicillium chrysogenum* are isolated worldwide and must be regarded as cosmopolitan. In many cases, conidia might 'land' closely to the location of production, but still many spores making into the higher air layers. There is indirect evidence that spores may be able to travel large distances through the air. For example, *Aspergillus sydowii conidia* have been suggested to travel over thousands of kilometers from the Sahara Desert to the Caribbean reefs.

Distribution in time is occurring as stress-resistant cells remain dormant at one location for an extended period, awaiting conditions that are more favorable for growth. Some ascospores (sexual spores) are extremely stress-resistant and dormant for very long periods. Other species show extended dormancy in a dried state. As microbial species are inherently variable, stress resistance varies between strains from the same species. For example, conidial heat resistance (D60) of various strains of the fungus *Paecilomyces variotii* ranged between 3.5 to 27.6 min. This intraspecific variation could have profound consequences on diagnostics, virulence, and antifungal treatment in clinical settings.

For conidial germination in most filamentous fungi, the presence of nutrients such as inorganic salts, sugars, and amino acids is required. The swelling phase of conidia is also called isotropic growth. Swollen conidia direct the growth to one side of the cell to grow in a polarized fashion, which leads to the formation of a germ tube (polarized growth). There is a notable drop in stress resistance during isotropic and polarized growth and genes expressed during these stages might represent novel targets for fungal infection.

**S5.3c**
**Investigating the link between pleomorphism and virulence in *Cryptococcus***

Kenya Fernandes[1], James Fraser[2], Dee Carter[1]
[1]School of Life and Environmental Sciences, University of Sydney, Australia
[2]School of Chemistry and Molecular Biosciences, University of Queensland, Australia

S5.3 Cellular pleomorphism and fungal virulence, September 22, 2022, 3:00 PM - 4:30 PM

Objectives: Fungal pathogens *Cryptococcus neoformans* and *Cryptococcus gattii* are responsible for hundreds of thousands of annual deaths in immunocompromised individuals. Considerable phenotypic variation is exhibited by strains in response to stresses encountered during host infection, including increased capsule and cell size, the release of shed capsule, and the production of giant (> 15 $\mu$M), micro (< 1 $\mu$M), and irregular cells. We aimed to investigate whether the production of these morphological variants is associated with virulence using two sets of strains. The first is a collection of diverse clinical isolates obtained from HIV/AIDS patients in Botswana with accompanying clinical data. The second is a collection of lineages derived from the *C. neoformans* type strain H99 with high genetic similarity but differing levels of virulence. Some lineages in this set possess a mutation in SGF29, which encodes a component of the SAGA histone acetylation complex that has previously been implicated in their hypervirulence.

Methods: Isolates were cultured under conditions that simulate stresses encountered *in vivo* (DMEM, 5% $CO_2$, 37°C) as these are known to enhance capsule production and induce cell size changes. Cells were counterstained with india ink, visualized by light microscopy, and phenotypes were scored. For clinical isolates, MLST analysis was performed to determine their relatedness. For H99 strains, *Galleria mellonella* larval infection assays, growth curves, and antifungal susceptibility testing was performed to confirm their relative virulence and growth profiles. Serial block face and regular scanning electron microscopy were used to investigate the internal morphology of the giant, micro, and irregular cells to confirm that they possess attributes of functional cells.

Results: Substantial pleomorphism was seen across both collections. In the clinical strain set, phenotypic variables fell into two groups associated with differing symptoms. The production of 'large' phenotypes was associated with a higher CD4 count and was negatively correlated with intracranial pressure indicators, suggesting that these are induced in early-stage infection. 'Small' phenotypes were associated with lower CD4 counts, negatively correlated with meningeal inflammation indicators, and positively correlated with intracranial pressure indicators, suggesting that they are produced later during infection and may promote proliferation and dissemination. Isolates possessing giant cells, microcells, and shed capsules were rare, but strikingly, they were associated with patient death.

In the H99 set, strains from hypervirulent lineages had larger average capsule size, greater variation in cell size, and increased production of microcells and shed capsules. Deletion of SGF29 in an intermediate virulence lineage substantially increased its production of microcells and released capsule, consistent with a switch to hypervirulence. SGF29 loss-of-function mutations were subsequently identified in clinical isolates and were found to be significantly correlated with patient death. Expansion of a TA repeat in the second intron of SGF29 in clinical isolates was positively correlated with cell and capsule size, suggesting it also affects Sgf29 function.

Conclusion: Our results extend the evidence for a link between pleomorphism and virulence, with a likely role for epigenetic mechanisms mediated by SAGA-induced histone acetylation.

**S5.3d**
**How mitochondrial complex I proteins in *Candida albicans* moderate phagocytosis and the production of pro-inflammatory cytokines in murine macrophages and dendritic cells**

Xiaodong She[1], Pengyi Zhang[3], Richard Calderone[2], Weida Liu[1], Dongmei Li[2]
[1]Institute of Dermatology, Chinese Academy of Medical Sciences (cams) and Peking Union Medical College (PUMC), Nanjing, China
[2]Georgetown University Medical Center, Department of Microbiology and Immunology, Washington, USA
[3]Sport Science Research Center, Shandong Sport University, Jinan, China

S5.3 Cellular pleomorphism and fungal virulence, September 22, 2022, 3:00 PM - 4:30 PM

Objectives: Inhibition of respiration in *Candida albicans* impairs its colonization in the host tissues and causes avirulence in a murine vascular candidiasis model. Accordingly, blockage of the mitochondrial electron transport chain (ETC) of *C. albicans* by respiratory inhibitors promotes phagocytosis by increasing exposure of glucan which could be due to the mannan reduction. In our model, we have reported that 85% mannan reduction in goa1Δ, a deletion mutant of an ETC Complex I (CI) regulator, oppositely decreased phagocytosis. To understand such a difference, we broaden our investigation with three CI respiratory subunit mutants, which are either fungal-specific (nuo1Δ and nuo2Δ) or broadly conserved subunits (ndh51Δ) for cell wall analysis and innate immune responses.

Methods: We characterized mutant cell wall defects in these mutants, then analyzed their respective survival in macrophages. Fungal internalization into macrophages was visualized under fluorescent microscopy and live-cell imaging and analyzed through flow cytometry analysis. Cytokine production in dendritic cells (DCs) infected by fungal cells was measured by xMAP technology and the transcriptional profiles of murine macrophages-infected by different mutants were compared.

Results: We find that phosphopeptidomannan (PPM) reduction in goa1Δ and nuo1Δ and phospholipomannan (PLM) reduction in nuo2Δ correlate with massive inhibition of cytokine. PPM loss in nuo1Δ or goa1Δ fails to promote phagocytosis but promotes opsonized neutrophil killing. The cause of PPM insufficiency results from reduced phosphorylation of the Cek1 MAPK in goa1Δ and nuo1Δ. In contrast other three mutants, phagocytosis and cytokine production of ndh51Δ more resemble WT cells, which have shown an ~ 30% glucan reduction due to a defective Mek1 MAPK response. The divergent immune responses to these CI mutants are shown at the transcriptional level in infected macrophages. We noted that those well-characterized host receptors such as dectins and TLR2/4 for PPM, PLM, and glucan ligands are not significantly affected at 1 h post-infection. However, the scavenger receptor CD36, integrin ICAM, and growth factor receptors are downregulated along with a generally downregulated endocytosis and antigen processing/presentation. In addition, the host metabolic processes, oxidative stress-induced senescence, apoptosis, and signaling pathways such as Ras1/Erk5, the cAMP/CREB, and TLR9 pathway, are each individually affected in the host cells.

Conclusion: We speculate that mitochondrial signals of fungal origin may also be sensed by the host immune cells to coordinate the immune responses together with cell replication and metabolism during the early stage of infection.