Understanding the function of the Pga15 family in *Candida albicans* pathobiology

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Background: The cell wall of the human pathogen *C. albicans* is considered to be an important structure. The inner layer of the *C. albicans* cell wall is composed of chitin and glucan polymers. The outer cell surface is composed of mannoproteins that may be involved in sensing and adapting to the surrounding environment, maintaining a robust cell wall or interactions with the host.

- The *C. albicans* genome has 115 predicted GPI proteins (Richard and Plaine, 2007), which includes 22 protein families.
- The Pga15 family include 3 uncharacterised proteins: Pga15, Pga41 and Pga42 found only in *C. albicans* and in *C. dubliniensis* .They have unknown function and no similarity to any characterised proteins.

Study Aims: understand the role of the Pga15 protein family through phenotypic characterisation of *pga15*Δ family mutants.

Results: Deletion of *PGA15* family members leads to growth-medium dependent altered hyphal growth phenotypes

*pga41*Δ as well as *pga42*Δ have statistically significant longer hyphae in comparison to the control strain after 1.5 hours incubation in 10% FCS (Fig 1a). After four hours' incubation, *pga42*Δ, *pga41*Δ*pga42*Δ formed longer hyphal cells compared to the control. (Fig 1b,2a). On the other hand, *pga15*Δ and *pga41*Δ formed statistically significantly longer hyphae in GlcNAc medium after two hours (Fig 1c), while *pga42*Δ had statistically significant shorter hyphae after two and four hours (Fig 1c,d;2b).

In neutral pH medium, *pga42*Δ formed statistically significant shorter hyphae in contrast to control at four and six hours (Fig 1 e,f). *pga41*Δ and *pga41*Δ*pga42*Δ formed longer hyphae after four hours incubation in neutral pH, and *pga15*Δ had statistically significantly longer hyphal cells at four and six hours compared to control (Fig 2c).

Biofilm formation and adhesion: *pga42*Δ and *pga41*Δ*pga42*Δ had statistically significantly higher biofilm formation over four and six hours in 10% FCS as well as RPMI (Fig 3). In addition, *pga42*Δ are more hydrophobic and adheres more to laminin compared to the control (data not shown).

Disruption of *pga15* family leads to changes in *C. albicans* cell wall ultrastructure

High pressure freezing TEM was used to examine the cell wall architecture. The cells were grown in 2 different media YPD and NGY. We found that there were changes in the cell wall ultrastructure between the mutants and the control and between different media (Fig 4).

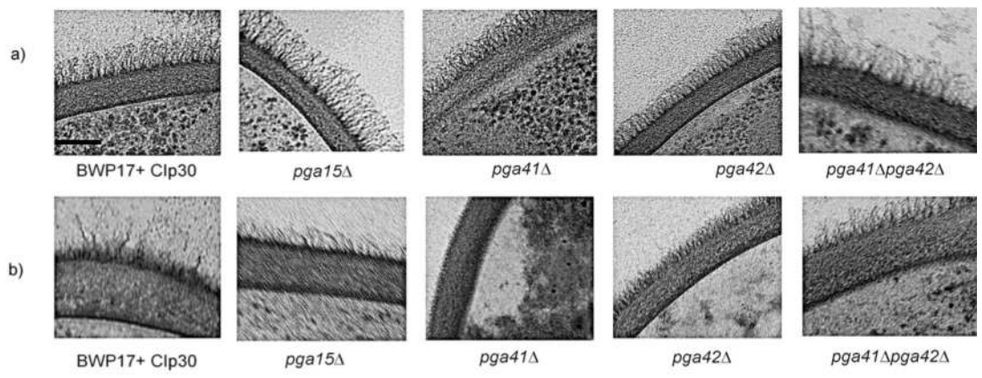

Fig 4 Fungal cell wall ultrastructure of *pga15*Δ family mutants
Electron micrographs of a) YPD-grown and b) NGY-grown cells. Scale bar represents 100 nm

Conclusions Absence of Pga15 family members impacts on hyphal growth, biofilm formation and cell wall architecture.

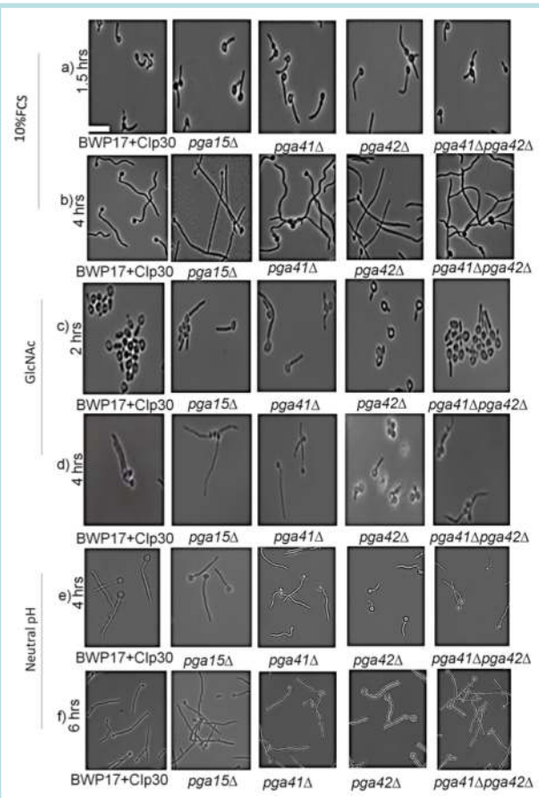

Fig 1: Hyphal induction in different media
Cells were grown overnight in YPD at 30Cº.
Hyphae were induced at 37Cº in 10% FCS
for a) 1.5 h, b) 4 h. In GlcNAc medium for c) 2 h, d) 4 h and at pH 7.0 for e) 4 h f) 6 h. Scale bar is 100µm.

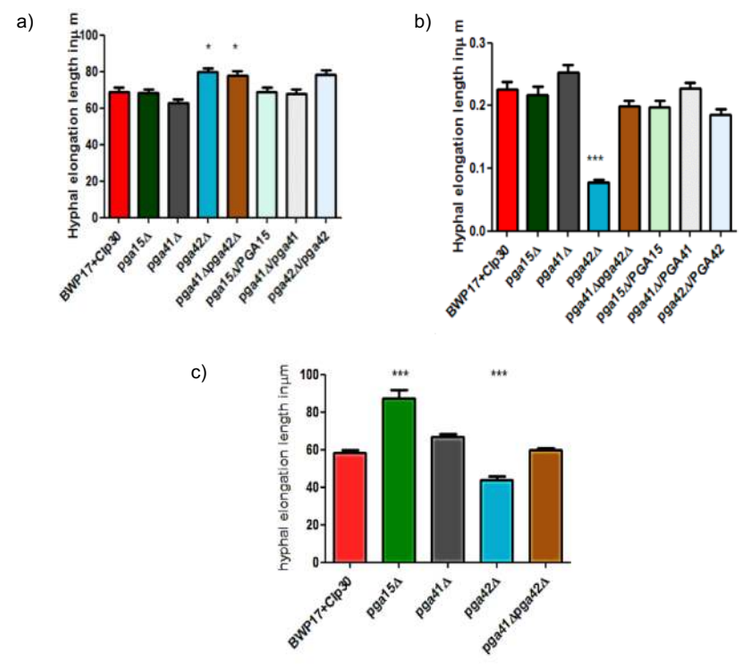

Fig 2: Disruption of Pga15 family impacts hyphal formation
Hyphal length measurement at a) 4h in 10% FCS b) at 4h in GlcNAc c) in neutral pH at 6h

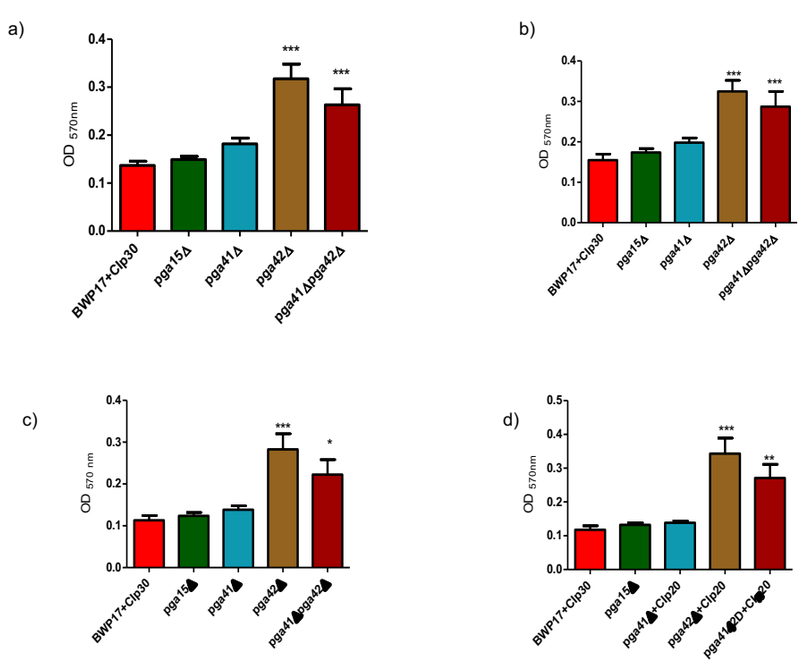

Fig 3: Biofilm biomass in 10% FCS and RPMI
Cells were grown overnight in NGY at 30Cº washed 2 times with PBS and 1x 10^6 of cells inoculated into RPMI or 10% FCS. Biofilm biomass was measured using crystal violet staining. RPMI medium a) after 4h, b) after 6 h. In10% FCS c) after 4 h, d) after 6 h

Richard ML & Plaine A (2007) Comprehensive analysis of glycosylphosphatidylinositol-anchored proteins in *Candida albicans*. *Eukaryot Cell* **6**: 119–133.