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Role of autophagy genetic variants for the risk of *Candida* infections

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Abstract

Candida albicans can cause candidemia in neutropenic and critically ill patients, and oropharyngeal candidiasis in HIV-positive patients with low $CD4^+$ counts. However, not all patients at risk develop *Candida* infections, and the genetic background of the patient might play a role in the susceptibility to infection. Autophagy mediates pathogen clearance and modulation of inflammation. The aim of this study was to assess the effect of genetic variation in the *ATG16L1* and *IRGM* autophagy genes on the susceptibility to candidemia and oropharyngeal candidiasis.

We assessed whether genetic variation in the *ATG16L1* and *IRGM* genes influences susceptibility to candidemia in a cohort of candidemia patients of both African and European origin. In addition, we assessed the effect of these polymorphisms for the susceptibility to oropharyngeal candidiasis in an HIV-positive cohort from Tanzania. Functional studies have been performed to assess the effect of the *ATG16L1* and *IRGM* genetic variants on cytokine production both *in vitro* and *in vivo*. The results indicate that *ATG16L1* variants modulate production of TNFα, but not other cytokines, while no effects were seen in the presence of *IRGM* polymorphisms. In addition, no

Conflicts of Interest

All authors declare no conflicts of interest.

significant associations between the SNPs in the *ATG16L1* and *IRGM* genetic variants and the incidence of candidemia or oropharyngeal candidiasis were identified.

In conclusion, despite moderate effects on the modulation of proinflammatory cytokine production, genetic variation in the autophagy genes *ATG16L1* and *IRGM* has a minor impact on the susceptibility to both mucosal and systemic *Candida* infections.

Introduction

Candida albicans is a dimorphic fungus that is commensal in immunocompetent individuals, colonizing the skin, gastrointestinal tract and oral and genital mucosa. However, under certain host immune defense conditions, *C. albicans* can become pathogenic, being the most common fungal pathogen in humans [1]. *C. albicans* can cause both mucosal and disseminated infections. Candidemia is a systemic infection with *Candida* spp., predominantly but not limited to *C. albicans*, that occurs mostly in patients that are immunocompromised due to neutropenia, in critically-ill patients with use of broad spectrum antibiotics, invasive surgery, or in patients receiving total parenteral nutrition [2, 3]. Moreover, other risk factors such as ethnic background might also play a role based on the genetic differences between individuals of European vs. African ancestry [4]. Oropharyngeal candidiasis (OPC) is a *Candida* mucosal infection that occurs very often as an opportunistic infection in HIV positive patients with a low $CD4^+$ count (<200 cells/μL) [5–7]. In the absence of proper treatment, the chance of spread of the infection to the bloodstream increases [8].

However, not all patients at risk develop *Candida* infections, and part of the differential susceptibility may be explained by the genetic makeup of the patients, as exemplified by several reports published previously [9–12]. Autophagy is a highly conserved housekeeping molecular process important for cellular development, maintenance, energy turnover and antigen presentation. The autophagy machinery enables the formation of double membrane vesicles leading to the sequestration of components of the cytoplasm into double membrane vesicles that ultimately fuse with the lysosome for degradation of the autophagosomal content [13]. Furthermore, autophagy is enabling the lysosomal digestion and antigen presentation of phagocytosed materials such as fungal and bacterial pathogens, including *Candida* spp. [14].

Previous studies have demonstrated that genetic variation in autophagy genes affect the modulation of inflammation by autophagy [15]. A genetic variant in *ATG16L1* (Thr300Ala, rs2241880) has been associated with susceptibility to Crohn's disease, with the *ATG16L1**300A conferring a higher risk [16–18]. Moreover, recent studies have shown that this *ATG16L1* polymorphism not only affects the capacity to execute autophagy [19], but also to modulate proinflammatory cytokine responses, especially IL-1β [20], and influences host defense against microorganisms such as mycobacteria [21].

Similarly, genetic polymorphisms in the *IRGM* (Immunity-related GTPase family M protein) gene are associated with Crohn's disease, including the SNPs rs13361189 and rs4958847 [22]. Interestingly, it has been shown that one of these *IRGM* SNPs, rs13361189,

is in perfect linkage disequilibrium with a 20 kb insertion/deletion polymorphism situated upstream of the *IRGM* promoter region. Consequently, this genetic variant modulates the expression of the *IRGM* gene [23]. Considering the data linking *ATG16L1* and *IRGM* SNPs with the modulation of immune responses by autophagy, it is compelling to assess whether these genetic variants could influence the susceptibility to fungal infections, and in particular oropharyngeal and/or disseminated candidiasis because of their functional effects on the autophagy machinery. Although in a recent study we were not able to identify a crucial role of autophagy in systemic *Candida* infections [24], no information is available on the role of autophagy in mucosal infection with *Candida.* Interestingly, *Candida albicans* is also one of the immunogens for developing anti-*Saccharomyces cerevisiae* antibodies (ASCA), which are regularly observed in patients with mucosal lesions in Crohn's disease [25, 26].

In order to determine the role of the genetic polymorphisms in two autophagy genes in the susceptibility to both systemic and mucosal infections with *Candida albicans*, we evaluated the frequency of the genotypes of three polymorphisms in the *ATG16L1* and *IRGM* genes in the predisposition to two different types of *Candida albicans* infections: oropharyngeal candidiasis in HIV positive patients and disseminated candidiasis or candidemia in intensive care unit patients. In order to support the genetic analysis, functional assays were performed with peripheral blood mononuclear cells bearing different *ATG16L1* and *IRGM* genotypes and serum cytokine measurements were conducted by comparing the genotypes for their ability to produce cytokines upon *Candida albicans* stimulation either *in vitro* or *in vivo*. Finally, a bioinformatic analysis was performed with the aim to further explain the possible functional consequences of the genetic variants in *ATG16L1* and *IRGM*.

Patients and Methods

HIV positive patients

The effect of the polymorphisms associated with the autophagy process in the predisposition to OPC was assessed in a group of 155 HIV-seropositive patients recruited at the Muhimbili National Hospital HIV-clinic in Dar-es-Salaam, Tanzania. The recruitment period took place between April 2007 and August 2008. The patients' clinical examination was performed by an independent physician according to the WHO clinical staging criteria [27]. The oral examination was routinely performed at each visit according to WHO rules [28]. Variations in color, size and shape of anatomical areas of the intraoral tissue were taken as clinical sings for OPC. The clinical manifestations of OPC in these patients were diverse, presenting a pseudomembranous candidiasis or a combination of pseudomembranous and erythematous, hyperplastic, or angular cheilitis. This cohort has been previously reported in genetic association studies by our group [29], and the study was approved by the Ethical Committee of the Hospital. Sampling was performed by firmly swabbing the lesion site with a sterile cotton wool swab. Immediate microbiological confirmation was performed, the samples were sent immersed in 10% potassium hydroxide (KOH). Within this cohort, 82 patients developed OPC, and 73 patients did not present with OPC.

Candidemia patients

Patients were enrolled after informed consent (or waiver as approved by the Institutional Review Board) at the Duke University Hospital (DUMC, Durham, NC, USA) and Radboud University Medical Center (RUMC, Nijmegen, The Netherlands). The study was approved by the Institutional Review Boards at each study center, and enrollment occurred between January 2003 and January 2009. To be included in the analysis of susceptibility to infection, infected subjects must have had at least one positive blood culture for a *Candida* species while hospitalized at the participating center. Non-infected controls must have been hospitalized with no history or evidence of candidemia/invasive candidiasis, or any invasive fungal infection. Non-infected controls were recruited from the same hospital wards as infected patients in such a way that comorbidities and clinical risk factors for infection would be similar between groups. The clinical characteristics of the patients have been previously reported in genetic association studies [9, 24, 30].

Genetic analysis

Genomic DNA was isolated from whole blood using standard procedures. Genotyping for the *ATG16L1* T300A (rs2241880) and the *IRGM* SNPS (rs13361189 and rs4958847) single nucleotide polymorphisms (SNPs) was performed by using the TaqMan single-nucleotide assay C_9095577_20, C_31986315_10 and C_1398968_10, respectively, on the 7300 ABI Real-Time polymerase chain reaction system (all from Applied Biosystems, CA, USA). Two of these polymorphisms (rs2241880 and rs4958847) were also part of the Sequenome (Sequenom MassARRAY®, Sequenom, San Diego, CA) analysis of the study of Smeekens at al (24). However, we chose to assess it in the present study through TaqMan technology in the candidemia cohort, due to the fact that the quality of DNA necessary for Sequenome analysis was not sufficient for the entire cohort of patients. The assessment of all three polymorphisms in the studies on mucosal forms of infections has not been presented elsewhere.

Cytokine stimulation assays

Peripheral blood mononuclear cells (PBMC) were isolated from 73 healthy volunteers by Ficoll-Paque gradient. Subsequently, stimulation with heat-killed *Candida albicans* blastoconidia was performed for 24 hours, 48 hours or 7 days. The stimulation time varied for each cytokine, being 24 hours for IL-1β, IL-6, IL-8 and TNFα, 48 hours for IFNγ and IL-10, and 7 days for IL-17. At the end of the incubation time, the above-mentioned cytokines were measured in the supernatants by ELISA (purchased from R&D systems Minneapolis, MN, or Sanquin Research, Amsterdam, The Netherlands). Comparisons of cytokine production between patients bearing the different *ATG16L1* or *IRGM* genotypes were performed.

Serum cytokine measurements

Cytokine concentrations of IL-6, IL-8 and IFNγ in plasma and serum samples obtained from infected patients from day 0 up to day 5 after initial positive blood culture were measured by Multiplex Fluorescent Bead Immunoassays (xMAP technology, Bio-Rad, Veenendaal, The

Netherlands) and a Bio-plex microbead analyzer (Luminex, Austin, TX, USA) according to the manufacturer's protocol.

Bioinformatic analysis

In order to predict the effect on the conformation of the ATG16L1 protein given the T300A amino acid change we used HOPE (http://www.cmbi.ru.nl/hope/), a next generation web server that performs automatic mutant analysis. No homology model was produced because no template with enough identity to the ATG16L1 sequence exists [31]. The HOPE server assessment was only possible for the *ATG16L1* SNP since it is the only SNP from this study that alters the amino acid sequence and the HOPE servers works exclusively in case of non-synonymous mutations and their effect on protein function.

Statistical analysis

For the genetic analysis, statistical comparisons of frequencies were made between infected and non-infected patients (with either OPC or candidemia) by performing the Chi-square test with SPSS version 20 (IBM SPSS Statistics for Windows Version 20.0. Armonk, NY: IBM Corp. For the analysis of the *in vitro* PBMC stimulation and *in vivo* serum cytokine measurements, a Kruskal-Wallis one-way ANOVA was performed with the use of GraphPad Prism version 4.00 (GraphPad Software, San Diego, CA, USA). Corrections for multiple testing have not been performed due to the absence of significant differences, and throughout the manuscript only uncorrected *P*-values are mentioned.

Results

Functional analysis of cytokine profiles

The cytokines measured in the in vitro assays are chosen based on their role in the anti-*Candida* immune response: TNFα, IL-1β, IL-6, IL-8 and IL-10 to monitor the response of monocytes, and IFNγ and IL-17 as T-cell derived cytokines to monitor the activation of the Th1 and Th17 responses, respectively. A significant difference in *C. albicans*-induced cytokine production was observed between cells isolated from individuals with different *ATG16L1* genotypes for production of TNFα ($P = 0.0039$) (Figure 1). However, no differences were observed in the production of IL-1β, IL-6, IL-8, IL-10, IFNγ and IL-17. In addition, the effect of the genotype of the *IRGM* gene on the *in vitro* cytokine production was evaluated. These analyses revealed that the only statistically significant effect of the rs13361189 genotype was observed for production of IL-8. However, *Candida* induced IL-8 production was not affected by the other SNP in *IRGM*. Furthermore, TNFα, IL-1β, IL-6, IL-10, IFNγ and IL-17 production were not affected by any of the two investigated SNPs in *IRGM*.

Bioinformatic Analysis using the HOPE server

We also performed a bioinformatic analysis using the HOPE next-generation server [31]. The output indicates that the change from a threonine into an alanine at position 300 of the ATG16L1 protein introduces changes in the amino acid size and hydrophobicity. The mutant residue is smaller and more hydrophobic than the wild-type residue. Moreover, the server predicted that the polymorphism would cause an empty space in the core of the

protein (or protein-complex). The change in the hydrophobicity between the wild-type and mutant-type amino acids would cause a loss of hydrogen bonds in the core of the protein (or protein-complex), and as a results it would disturb correct folding (Supplementary figure 1).

Oropharyngeal candidiasis

The distribution of the *ATG16L1* and *IRGM* polymorphisms in the HIV-positive patient groups with or without OPC is shown in Table 1. No statistically significant differences were observed. Since the $CD4^+$ count is considered as a confounder, we stratified the study subjects based on their $CD4^+$ counts. No significant association was observed in either $CD4^+$ count group (Table 2).

Disseminated Candidiasis

The intergroup comparison between the Dutch RUNMC and North-American Caucasian DUMC controls and patients revealed a similar genetic distribution of the genotyped SNPs, which allowed the groups to be merged into one group of subjects of European descent (data not shown). Genetic distribution of the *ATG16L1* and *IRGM* polymorphisms in the studied patient groups is shown in Table 3. No significant associations of polymorphisms in *ATG16L1* and *IRGM* with susceptibility to candidemia were revealed ($P>0.05$).

Serum samples collected from patients with bloodstream *Candida* spp. infections during the first 5 days after initial positive blood culture were measured for concentrations of IL-6, IL-8 and IFNγ. The results are shown in Figure 2: IL-6, IL-8, and IFNγ circulating concentrations were not affected by any of the SNPs in *ATG16L1* and *IRGM.* Other cytokines such as TNFα, IL-1β, IL-10 and IL-17 were not detectable in the serum samples (data not shown).

Discussion

In the current study, we evaluated the effect of polymorphisms in the autophagy genes *ATG16L1* and *IRGM* [15, 23] on the susceptibility to OPC and candidemia, respectively. Upon stimulation with *Candida* blastoconidia, the *in-vitro* cytokine production capacity of primary immune cells with different *ATG16L1* genotypes demonstrated a lower production of TNFα by cells bearing the ATG16L1 300A allele. On the other hand, the variant allele of *IRGM* rs13361189 moderately increased IL-8 production under similar conditions. Despite these differences, the genotype of the *ATG16L1* and *IRGM* SNPs did not affect the susceptibility of patients to either OPC or systemic candidiasis.

Genetic variation in genes coding for the autophagy regulatory proteins are known to alter the clearance of intracellular bacteria [23]. The SNP rs2241880 of *ATG16L1* causes a partial loss of function in the ATG16L1 protein, inhibiting the *Salmonella*-induced autophagy process, which in turn results in reduced clearance of the microorganisms in human epithelial cells [15]. In addition, a polymorphism in the promoter region of the *IRGM* gene conferred an increased susceptibility to tuberculosis [32]. 21 different mouse IRG genes are located on chromosomes 11 and 18, and have been demonstrated to have a very powerful effect on the clearance of intracellular pathogens such as *Listeria monocytogenes, Toxoplasma gondii* [33, 34], *Mycobacterium tuberculosis* [35], *Salmonella typhimurium* and

Chlamydia trachomatis [34]. Furthermore, genetic variation in both *ATG16L1* and *IRGM* genes is associated with a higher susceptibility to Crohn's disease [16–18, 22]. Conversely, the role of these genetic polymorphisms on the susceptibility to fungal pathogen has yet to be studied. Therefore, we have investigated whether SNPs in *ATG16L1* and *IRGM* are related to the propensity to develop oropharyngeal and/or systemic *Candida* infections.

The effect of the T300A polymorphism on ATG16L1 function is the best characterized among the three SNPs evaluated in this study. The loss of function predicted with the HOPE server was evident on TNFα production, with a decrease paralleling the allelic dosage. This polymorphism is known to reduce autophagy [15], which is supported by a previous study [36]. Nevertheless, genetic variation in the *ATG16L1* gene did not influence the susceptibility to either oropharyngeal or systemic candidiasis.

In contrast to the *ATG16L1* SNP, the *IRGM* polymorphisms evaluated in this study do not affect the IRGM protein sequence or structure, but modulate *IRGM* gene expression. McCarroll and colleagues demonstrated that *IRGM* haplotypes differentially affected gene expression in different cell types, and that the gene expression levels affected the efficiency of autophagy for eradicating intracellular bacteria [23]. Despite the possible consequences of the variation in the two *IRGM* SNPs, the genotype of either polymorphism did not affect the predisposition to oropharyngeal or systemic *Candida* infection.

The observation that the investigated SNPs in *ATG16L1* and *IRGM* are not associated with increased susceptibility to *Candida* infections, but are involved in the pathogenesis of Crohn's disease, may suggest that the link between *Candida* colonization and Crohn's disease, through the presence of ASCA antibodies, is not mediated by autophagy. However, one has to realize that the mechanisms underlying *Candida* colonization vs. *Candida* infection are largely different, as recently shown by our group [32]. Therefore, although autophagy appears not to be involved in susceptibility to *Candida* infection as demonstrated by the present study, it still could be associated with *Candida* colonization. Future studies are warranted to assess this aspect.

Autophagy-mediated pathogen clearance is an important process mainly for host defense against intracellular bacteria such as mycobacteria or *Salmonella typhimurium*. Whereas of the role of autophagy in the clearance of extracellular bacteria has been demonstrated [37], the role of autophagy for anti-*Candida albicans* host defense remains unclear. The lack of association of the SNPs in *ATG16L1* and *IRGM* with susceptibility to *Candida* infections implies that host defense against *Candida* infections does not depend on these two genetic variants in autophagy genes. The results of the present study are strengthened by an additional report that showed that although *Candida* was able to induce autophagy *in vitro*, this was not necessary for host defense against disseminated candidiasis in mice [24]. Moreover, a set of polymorphisms in autophagy genes did not modulate susceptibility to candidemia [24]. The complementary results of these investigations strongly argue that autophagy is a redundant mechanism for host defense against both systemic and mucosal *Candida* infections.

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References

1. Webb BC, Thomas CJ, Willcox MDP, et al. *Candida*-associated denture stomatitis. Aetiology and management: A review. Part 1. Factors influencing distribution of *Candida* species in the oral cavity. Australian Dental Journal. 1998; 43(1):45–50. [PubMed: 9583226]
2. Perlroth J, Choi B, Spellberg B. Nosocomial fungal infections: epidemiology, diagnosis, and treatment. Medical Mycology. 2007; 45(4):321–346. [PubMed: 17510856]
3. Spellberg B. Novel insights into disseminated candidiasis: Pathogenesis research and clinical experience converge. Plos Pathogens. 2008; 4(2):e38. [PubMed: 18282100]
4. Barreiro LB, Ben-Ali M, Quach H, et al. Evolutionary Dynamics of Human Toll-Like Receptors and Their Different Contributions to Host Defense. PLoS genetics. 2009:e1000562. [PubMed: 19609346]
5. Goodman DS, Teplitz ED, Wishner A, et al. Prevalence of cutaneous disease in patients with acquired-immunodeficiency-syndrome (AIDS) or aids-related complex. Journal of the American Academy of Dermatology. 1987; 17(2):210–220. [PubMed: 2957397]
6. Phelan JA, Saltzman BR, Friedland GH, et al. Oral findings in patients with acquired-immunodeficiency-syndrome. Oral Surgery Oral Medicine Oral Pathology Oral Radiology and Endodontics. 1987; 64(1):50–56.
7. Feigal DW, Katz MH, Greenspan D, et al. The prevalence of oral lesions in hiv-infected homosexual and bisexual men - 3 San-Francisco epidemiologic cohorts. Aids. 1991; 5(5):519–525. [PubMed: 1863403]
8. Degregorio MW, Lee WMF, Ries CA. *Candida* infections in patients with acute-leukemia - ineffectiveness of nystatin prophylaxis and relationship between oropharyngeal and systemic candidiasis. Cancer. 1982; 50(12):2780–2784. [PubMed: 6958353]
9. Plantinga TS, Johnson MD, Scott WK, et al. Toll-like Receptor 1 Polymorphisms Increase Susceptibility to Candidemia. Journal of Infectious Diseases. 2012; 205(6):934–943. [PubMed: 22301633]
10. Plantinga TS, van der Velden W, Ferwerda B, et al. Early Stop Polymorphism in Human DECTIN-1 Is Associated with Increased *Candida* Colonization in Hematopoietic Stem Cell Transplant Recipients. Clinical Infectious Diseases. 2009; 49(5):724–732. [PubMed: 19614557]
11. Ferwerda B, Ferwerda G, Plantinga TS, et al. Human Dectin-1 Deficiency and Mucocutaneous Fungal Infections. New England Journal of Medicine. 2009; 361(18):1760–1767. [PubMed: 19864674]
12. Glocker EO, Hennigs A, Nabavi M, et al. A Homozygous *CARD9* Mutation in a Family with Susceptibility to Fungal Infections. New England Journal of Medicine. 2009; 361(18):1727–1735. [PubMed: 19864672]
13. Stromhaug PE, Klionsky DJ. Approaching the molecular mechanism of autophagy. Traffic. 2001; 2(8):524–531. [PubMed: 11489210]
14. Nicola AM, Albuquerque P, Martinez LR, et al. Macrophage autophagy in immunity to *Cryptococcus neoformans* and *Candida albicans*. Infection and Immunity. 2012:3065–3076. [PubMed: 22710871]
15. Kuballa P, Huett A, Rioux JD, et al. Impaired Autophagy of an Intracellular Pathogen Induced by a Crohn's Disease Associated *ATG16L1* Variant. Plos One. 2008; 3(10):e3391. [PubMed: 18852889]
16. Hampe JA, Franke A, Rosenstiel P, et al. Genome-wide association scan of nonsynonymous SNPs identifies a susceptibility variant for Crohn disease in *ATG16L1*. Nature Genetics. 2007; 39(2): 207–211. [PubMed: 17200669]

17. Rioux JD, Xavier RJ, Taylor KD, et al. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. Nature Genetics. 2007; 39(5):596–604. [PubMed: 17435756]
18. Franke A, Balschun T, Karlsen TH, et al. Replication of signals from recent studies of Crohn's disease identifies previously unknown disease loci for ulcerative colitis. Nature Genetics. 2008; 40(6):713–715. [PubMed: 18438405]
19. Cooney R, Baker J, Brain O, et al. NOD2 stimulation induces autophagy in dendritic cells influencing bacterial handling and antigen presentation. Nature Medicine. 2010; 16(1):90–97.
20. Plantinga TS, Crisan TO, Oosting M, et al. Crohn's disease-associated *ATG16L1* polymorphism modulates pro-inflammatory cytokine responses selectively upon activation of NOD2. Gut. 2011; 60(9):1229–1235. [PubMed: 21406388]
21. Glubb DM, Gearry RB, Barclay ML, et al. *NOD2* and *ATG16L1* polymorphisms affect monocyte responses in Crohn's disease. World Journal of Gastroenterology. 2011; 17(23):2829–2837. [PubMed: 21734790]
22. Parkes M, Barrett JC, Prescott NJ, et al. Sequence variants in the autophagy gene *IRGM* and multiple other replicating loci contribute to Crohn's disease susceptibility. Nature Genetics. 2007; 39(7):830–832. [PubMed: 17554261]
23. McCarroll SA, Huett A, Kuballa P, et al. Deletion polymorphism upstream of IRGM associated with altered *IRGM* expression and Crohn's disease. Nature Genetics. 2008; 40(9):1107–1112. [PubMed: 19165925]
24. Smeekens SP, Malireddi RK, Plantinga TS, et al. Autophagy is redundant for the host defense against systemic *Candida albicans* infections. Eur J Clin Microbiol Infect Dis. 2013 In Press.
25. Standaert–Vitse A, Jouault T, Vandewalle P, et al. *Candida albicans* Is an Immunogen for Anti–*Saccharomyces cerevisiae* Antibody Markers of Crohn's Disease. Gastroenterology. 2006 May; 130(6):1764–1775. [PubMed: 16697740]
26. Quinton J-F, Sendid B, Reumaux D, et al. Anti-*Saccharomyces cerevisiae* mannan antibodies combined with antineutrophil cytoplasmic autoantibodies in inflammatory bowel disease: prevalence and diagnostic role. Gut. 1998; 42(6):788–791. [PubMed: 9691915]
27. Castro KG, Ward JW, Slutsker L, et al. 1993 Revised classification-system for HIV- infection and expanded surveillance case-definition for aids among adolescents and adults (reprinted from mmwr, vol 41, pg rr 17, 1992). Clinical Infectious Diseases. 1993; 17(4):802–810.
28. Kramer IRH, Pindborg JJ, Bezroukov V, et al. Guide to epidemiology and diagnosis of oral mucosal diseases and conditions. Community Dentistry and Oral Epidemiology. 1980; 8(1):1–26. [PubMed: 6929240]
29. Plantinga TS, Hamza OJM, Willment JA, et al. Genetic Variation of Innate Immune Genes in HIV-Infected African Patients With or Without Oropharyngeal Candidiasis. Jaids-Journal of Acquired Immune Deficiency Syndromes. 2010; 55(1):87–94.
30. Rosentul DC, Plantinga TS, Oosting M, et al. Genetic Variation in the Dectin-1/CARD9 Recognition Pathway and Susceptibility to Candidemia. Journal of Infectious Diseases. 2011; 204(7):1138–1145. [PubMed: 21881131]
31. Venselaar H, Te Beek TA, Kuipers RK, et al. Protein structure analysis of mutations causing inheritable diseases. An e-Science approach with life scientist friendly interfaces. BMC Bioinformatics. 2010; 11:548. [PubMed: 21059217]
32. Che NY, Li S, Gao TJ, et al. Identification of a novel *IRGM* promoter single nucleotide polymorphism associated with tuberculosis. Clinica Chimica Acta. 2010; 411(21–22):1645–1649.
33. Collazo CM, Yap GS, Sempowski GD, et al. Inactivation of LRG-47 and IRG-47 reveals a family of interferon gamma-inducible genes with essential, pathogen-specific roles in resistance to infection. Journal of Experimental Medicine. 2001; 194(2):181–187. [PubMed: 11457893]
34. Taylor GA, Feng CG, Sher A. P47 GTPases: Regulators of immunity to intracellular pathogens. Nature Reviews Immunology. 2004; 4(2):100–109.
35. MacMicking JD, Taylor GA, McKinney JD. Immune control of tuberculosis by IFN-gamma-inducible LRG-47. Science. 2003; 302(5645):654–659. [PubMed: 14576437]
36. Flicek P, Ahmed I, Amode MR, et al. Ensembl 2013. Nucleic Acids Research. 2013; 41(D1):D48–D55. [PubMed: 23203987]

37. Crisan TO, Plantinga TS, van de Veerdonk FL, et al. Inflammasome-Independent Modulation of Cytokine Response by Autophagy in Human Cells. Plos One. 2011; 6(4):e18666. [PubMed: 21490934]

38. Nakagawa I, Amano A, Mizushima N, et al. Autophagy defends cells against invading group a Streptococcus. Science. 2004; 306(5698):1037–1040. [PubMed: 15528445]

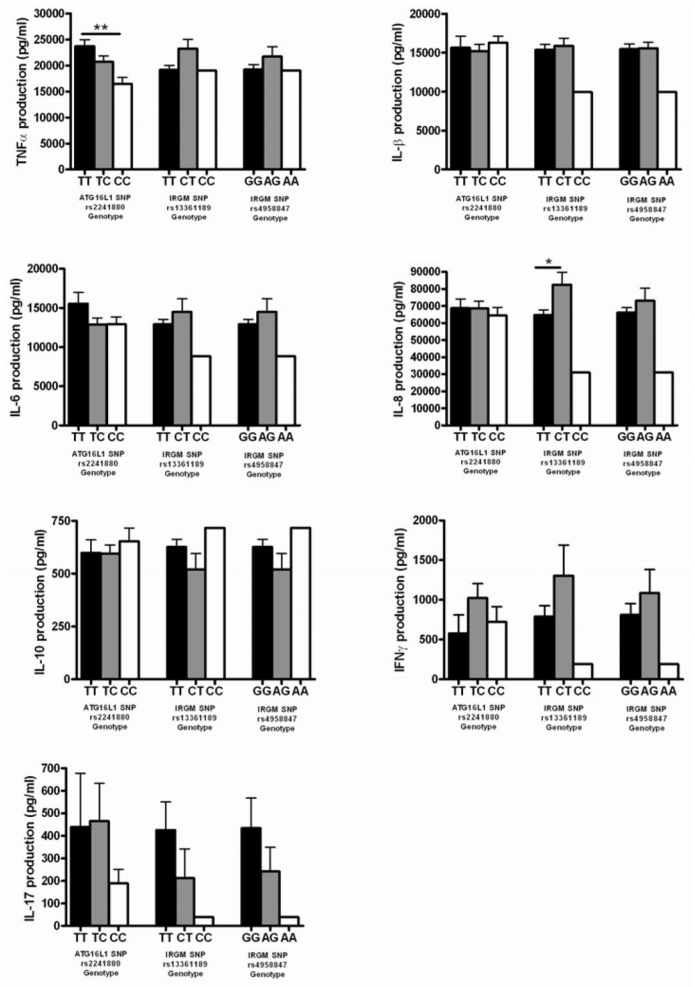

Figure 1.

Functional analysis on the stimulation of peripheral blood mononuclear cells (PBMCs) with heat-killed *C. albicans* blastoconidia. Cytokine production capacity of TNFα, IL-1β, IL-6, IL-8, and IFNγ was compared between the cells obtained from healthy volunteers bearing wild-type or variant *ATG16L1* or *IRGM* SNPs. Data are presented as means ± SEM, * $p < 0.05$. For the *ATG16L1* rs2241880 polymorphism the antisense nucleotides are depicted.

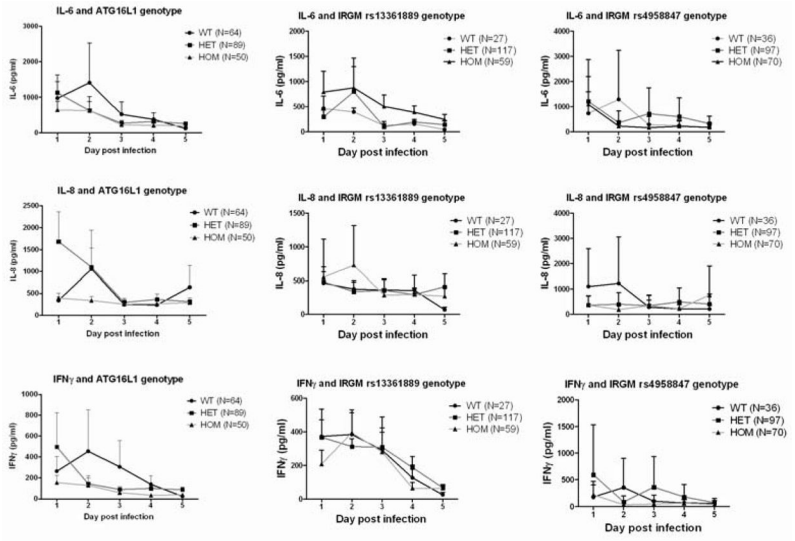

Figure 2.

Cytokine concentrations of IL-6, IL-8 and IFNγ in plasma and serum samples from infected patients from day 0 up to day 5 after initial positive blood culture correlated with the *ATG16L1* or *IRGM* genotype. WT: wild-type, HET: heterozygous. HOM: homozygous. Data are presented as means ± SEM, * $p < 0.05$.

Table 1

Frequencies of the genotype of the *ATG16L1* SNP number rs2241880 and the *IRGM* SNPs number rs13361189 and rs4958847 and the incidence of oropharyngeal candidiasis in HIV positive patients from Tanzania.

SNP	OPC	TT* (%)	TC* (%)	CC* (%)	*P*-value
***ATG16L1* rs2241880**	**Non-Infected (N = 73)**	40 (54.79)	29 (39.72)	4 (5.48)	0.287
	Infected (N= 81)	56 (69.14)	21 (25.93)	4 (4.94)	
***IRGM* rs13361189**	**OPC**	**CC (%)**	**CT (%)**	**TT (%)**	0.818
	Non-Infected (N = 68)	14 (20.59)	36 (52.94)	18 (26.47)	
	Infected (N= 77)	17 (22.08)	35 (45.46)	25 (32.47)	
***IRGM* rs4958847**	**OPC**	**AA (%)**	**AG (%)**	**GG (%)**	0.497
	Non-Infected (N = 71)	14 (19.72)	39 (54.93)	18 (25.35)	
	Infected (N= 82)	22 (26.83)	42 (51.22)	18 (21.95)	

Oropharyngeal candidiasis= OPC

*For the *ATG16L1* rs2241880 polymorphism the antisense nucleotides are depicted.

Table 2

Minor Allele Frequencies of the *ATG16L1* SNP number rs2241880 and the *IRGM* SNPs number rs13361189 and rs4958847 stratified by $CD4^+$ count in a cohort of HIV positive patients from Tanzania.

$CD4^+$ T cell count (minor allele frequency (%) OPC positive-OPC negative)				
SNP	**<100 (N=40)**	**100–200 (N=81)**	**200–300 (N=26)**	**>300 (N=5)**
***ATG16L1* rs2241880**	16.67%-14.29%	33.33%-28.89%	20.00%-18.75%	0%–30%
***IRGM* rs13361189**	64.52%-50%	50%–51.22%	40%–53.33%	75%–80%
***IRGM* rs4958847**	59.09%-50%	40.28%–46.67%	35%–63.33%	75%–87.5%

Oropharyngeal candidiasis= OPC

Table 3

Frequencies of the genotype of the *ATG16L1* SNP number rs2241880 and the *IRGM* SNPs number rs13361189 and rs4958847 and the incidence of candidemia in a cohort of hospitalized patients of African-American and European descent.

		African-American					European descent			
***ATG16L1* rs2241880**	**Candidemia**	**TT* (%)**	**TC* (%)**	**CC* (%)**	***P*-value**	**Candidemia**	**TT* (%)**	**TC* (%)**	**CC* (%)**	***P*-value**
	Non-Infected (N = 52)	28 (53.85)	18 (34.62)	6 (11.54)	0.303	**Non-Infected (N = 149)**	47 (31.54)	71 (47.65)	31 (20.81)	0.090
	Infected (N= 62)	25 (40.32)	30 (48.39)	7 (11.29)		**Infected (N= 168)**	35 (20.83)	90 (53.57)	43 (25.59)	
***IRGM* rs13361189**	**Candidemia**	**CC (%)**	**CT (%)**	**TT (%)**	0.456	**Candidemia**	**CC (%)**	**CT (%)**	**TT (%)**	0.761
	Non-Infected (N = 52)	7 (13.46)	24 (46.15)	21 (40.38)		**Non-Infected (N = 153)**	3 (1.96)	29 (18.95)	121 (79.08)	
	Infected (N= 62)	14 (22.58)	26 (41.94)	22 (35.48)		**Infected (N=165)**	5 (3.03)	34 (20.61)	126 (76.36)	
***IRGM* rs4958847**	**Candidemia**	**GG (%)**	**AG (%)**	**AA (%)**	0.392	**Candidemia**	**GG (%)**	**AG (%)**	**AA (%)**	0.719
	Non-Infected (N = 52)	20 (38.46)	25 (48.08	7 (13.46)		**Non-Infected (N = 149)**	109 (73.15)	36 (24.16)	4 (2.68)	
	Infected (N= 60)	19 (31.67)	27 (45.00)	14 (23.33)		**Infected (N= 168)**	118 (70.24)	43 (25.59)	7 (4.17)	

Oropharyngeal candidiasis= OPC

*For the *ATG16L1* rs2241880 polymorphism the antisense nucleotides are depicted.