Candida albicans is the most common human fungal pathogen causing mucosal and systemic infections. However, human antifungal immunity remains poorly defined. Here by integrating transcriptional analysis and functional genomics, we identified Candida-specific host defence mechanisms in humans. Candida induced significant expression of genes from the type I interferon pathway in human peripheral blood mononuclear cells. This unexpectedly prominent role of type I interferon pathway in anti-Candida host defence was supported by additional evidence. Polymorphisms in type I interferon genes modulated Candida-induced cytokine production and were correlated with susceptibility to systemic candidiasis. In in vitro experiments, type I interferons skewed Candida-induced inflammation from a Th17 response towards a Th1 response. Patients with chronic mucocutaneous candidiasis displayed defective expression of genes in the type I interferon pathway. These findings indicate that the type I interferon pathway is a main signature of Candida-induced inflammation and has a crucial role in anti-Candida host defence in humans.
**Candida albicans** is a commensal microorganism that inhabits human skin and mucosa. *Candida* can be isolated from up to 70% of the general population at any given moment, and it is believed that all individuals have been colonized with *Candida* at a some point. Although it is a commensal organism, *C. albicans* can also cause mucosal and systemic infections, especially in immunocompromised hosts. Despite the availability of novel classes of antifungal agents (for example, azoles, echinocandins), mortality due to systemic candidiasis, the fourth most common form of sepsis, reaches 37–44% (refs 3,6,7). In addition, vaginal and oral candidiasis can also occur in healthy or only mildly immunocompromised individuals, with up to 5% of women in the general population suffering from recurrent vulvovaginal candidiasis.

Despite the introduction of modern antifungal drugs and intensive care facilities, progress in improving the outcome of Candida infections in the last decade has been disappointing. Given this lack of progress, it is currently believed that only adjuvant immunotherapy will be able to further reduce the burden of morbidity and mortality caused by *C. albicans* infections. Understanding the host defence pathways involved in candidiasis is therefore crucial for identifying novel targets for immunotherapy. To date, all investigations aimed at identifying antifungal host defence mechanisms in humans have relied on candidate-target approaches that are based on biologic plausibility of hypotheses extracted from *in vitro* or animal studies. Although this approach has been fruitful in confirming important pathways of antifungal defence, it has lacked the power to provide a hierarchy of the importance of these pathways, and to identify potentially novel and unexpected host defence mechanisms against Candida.

In the present study, we have taken an alternative, unbiased approach to this biological problem. Using a combination of transcriptional analysis and systems biology, we have stimulated human primary cells with the fungus, identifying *Candida*-specific transcription profiles in human immune cells. Although *C. albicans* induced the transcription of multiple inflammatory gene sets commonly stimulated by other inflammatory stimuli, it also specifically induced several additional gene sets, among which we identified an unexpectedly strong type I interferon (IFN) signature profile. We confirmed the importance of the type I IFN pathway for anti-*Candida* host defence in humans through immunological and genetic studies in both healthy volunteers and in patients with systemic candidiasis or suffering from chronic mucocutaneous candidiasis (CMC).

**Results**

*C. albicans* induces a type I IFN response. We measured genome-wide transcriptional profiles in peripheral blood mononuclear cells (PBMCs) from healthy volunteers upon stimulation with *C. albicans*, as well as with inflammatory stimuli unrelated to fungal pathogens: *Escherichia coli*-derived lipopolysaccharide (LPS), *Borrelia burgdorferi*, *Mycobacterium tuberculosis* (MTB) and RPMI culture medium as a control. Measurements were performed at 4 and 24 h. A total of 693 transcripts that showed >2-fold higher expression compared with corresponding RPMI stimulation were selected (Benjamini and Hochberg’s (BH)-adjusted P < 0.05). K-means analysis identified profile clusters that indicated shared and specific genes that were differentially expressed in response to each stimulus. We grouped shared genes into signatures representing common early response genes (clusters 1, 6, 14, 27), common late response genes (clusters 2, 3) and common inflammatory genes induced by all stimulations (clusters 9, 21, 25) (Fig. 1a and Supplementary Figs S1–S3).

More importantly, to identify a set of discriminatory features (from the 693 differentially expressed transcripts) that would be most informative of the state of *Candida* stimulation and could distinguish *Candida* stimulation from the other inflammatory stimuli, we utilized information gain (Kullback–Leibler divergence/relative entropy) feature selection, to reduce the dimensionality of the feature space. We performed 10-fold cross-validation that stratifies the data into 10 random partitions in which each (in turn) is held out for testing and the remainder for training a Bayesian classifier. Class prediction was evaluated by computing the area under the receiver–operating characteristic curve, which assesses true- and false-positive rates. A 101-transcript (95-gene) *Candida*-response signature (Fig. 1b and Supplementary Table S1) was defined using this supervised learning approach for class prediction, achieving an area under the receiver–operating characteristic curve of 97.8%, which would indicate an excellent classifier performance with very high discriminative value. Principal component analysis of the *Candida*-response signature further demonstrated good class separation on the basis of *Candida* status and stimulation duration (Fig. 1c). As a set, the 101 features collectively identify expression signatures that are informative of *Candida* stimulation across the 4, 24 h or both time points that sufficiently distinguish *Candida* from the rest. Enrichment analysis for the 101 transcript *Candida*-response signature revealed a striking overrepresentation of the IFN signalling pathway (P = 3.8 × 10^{-3}) (Fig. 1d and Supplementary Table S2). This set of genes was strongly induced at both 4 and 24 h of *Candida* stimulation and establishes a definitive *Candida*-response signature distinct from that observed with LPS, MTB or *Borrelia* stimulation.

Type I IFN SNPs modulate susceptibility to candidemia. To assess whether the type I IFN genes specifically induced by *C. albicans* in human leukocytes are indeed involved in antifungal host defence, we investigated whether common polymorphisms in a subset of these genes influence susceptibility to systemic *Candida* infections. From a cohort of 217 Caucasian patients with candidemia, we had Immunochip genotyping data available and 11 genes of the 101-transcript (95-gene) data set were covered by the Immunochip array (Supplementary Table S3). We tested these 11 genes for association by taking immunochip data on 12,228 non-candidemia Caucasian healthy volunteers as controls. Analysis of the loci associated with these 11 genes revealed significant association of genetic variation with susceptibility to systemic candidiasis in four regions: CCL8 SNP 1kg_17_29697448 (P = 0.00069), STAT1 SNP rs16833172 (P = 0.0042), SP110 SNP rs3769845 (P = 0.012) and PSMB8 SNP rs3198005 (P = 0.01) (Fig. 2a–d; Supplementary Figs S1–S3, clusters 10, 11, 20, 11, respectively) (Supplementary Table S3). After applying a stringent Bonferroni correction for multiple testing, the association between susceptibility to candidemia and STAT1 and CCL8 remained significant. This finding confirms that the *C. albicans*-specific genes we identified in our transcriptional arrays have an important role in host defence against systemic fungal infections. We also attempted to assess whether the polymorphisms in these genes also modified disease severity. However, only 51 patients fulfilled the criteria of severe candidiasis based on clinical scores and persistence of positive blood cultures, and this number was too low for allowing the identification of significant differences between genetic variation and disease severity (disseminated disease, persistent fungemia and mortality at 14 or 30 days) (data not shown).

Type I IFN SNPs modulate *Candida*-induced cytokine responses. In an additional approach to validate the role of the type I IFN pathway for host defence against *Candida*, we isolated...
PBMCs from an additional group of 74 healthy volunteers and stimulated the cells with the fungus. Genetic variation in 11 type I IFN pathway genes present on the Immunochip was correlated with C. albicans-induced release of tumour-necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-8, IL-6, IL-10, IFN-γ and IL-17. In contrast to other microbial stimuli such as LPS and MTB, C. albicans induced higher levels of TNF-α, IL-1β and IL-10 production, and lower levels of IL-8 and IL-6 in human PBMCs (Supplementary Fig. S4). The correlation of cytokine concentrations with genotypes at tag single nucleotide polymorphisms (SNPs) of Candida-induced IFN pathway genes revealed significant associations at IRF1 and STAT1 regions (Fig. 3a–d) in which IRF1 SNPs were associated with TNF-α (P = 0.0028) and IL-10 levels (P = 0.0024), whereas STAT1 SNPs were associated with TNF-α (P = 0.001) and IL-6 levels (P = 0.0002). Consistent with the specificity of the type I IFN pathway for Candida stimulation, the association was significant with cytokine levels induced by Candida, but not by LPS or MTB stimulation (Supplementary Fig. S5).

Type I IFNs skew cytokine responses from Th17 towards Th1. To investigate the importance of type I IFN signalling in host defence against Candida, PBMCs from healthy individuals were stimulated with heat-killed C. albicans. In line with the transcriptional profile described above (Fig. 1), IFN-β could be detected in cell culture supernatants only after C. albicans stimulation. In contrast, neither live (Fig. 4a), nor heat-killed C. albicans induced any detectable IFN-α production.
We next assessed the role of type I IFN pathways in the induction of cytokines by *C. albicans*. When PBMCs were stimulated with heat-killed *C. albicans* in the presence of an antibody against IFN-α/β/ω receptor 1 (IFNAR), IFNγ production was significantly lower compared with PBMCs stimulated in the presence of a control antibody. Blocking of IFNAR did not influence the production of the monocyte-derived cytokines IL-ß or TNF-α (Fig. 4b). Conversely, adding IFN-α or IFN-β to *Candida*-stimulated cells increased IFN-γ production, while significantly decreasing IL-17 and IL-22 production (Fig. 4c). Together these data indicate that type I IFNs (in the context of infection, most likely IFN-β) modulate the immune reaction induced by *C. albicans*, skewing host defence towards a Th1 response.

**STAT1 mutations cause reduced expression of type I IFN genes.** Patients with autosomal-dominant CMC have increased susceptibility to mucosal fungal infections and often carry gain-of-function mutations in *STAT1* (refs 15,16), which lead to STAT1 hyperphosphorylation, that in turn likely makes STAT1 unavailable for forming heterodimers with other STAT molecules17. In co-cultures of dendritic cells (DCs) and T cells from CMC patients, this led to defective Th1 and Th17 responses (Supplementary Fig. S6). To validate the role of *Candida*-specific genes in human antifungal mucosal defence, we assessed the expression of these genes in cells from CMC patients. PBMCs from healthy controls and CMC patients were stimulated with RPMI, *E. coli*-derived LPS, heat-killed *C. albicans*, or heat-killed MTB. The expression of type I IFN pathway genes (including

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**Figure 2 | SNPs of type I IFN-responsive genes are associated with susceptibility to Candidemia.** Regional association plots, at (a) STAT1 region (b) CCL8/CCL13 region (c) SP110 region and (d) PSMB8 region, showing chromosome position in the x axis and the log of logistic P-values for each SNP in the y axis. Blue diamond indicates the top P-value; whereas orange and yellow diamonds indicates R² ranges of 0.6–0.8 and 0.4–0.59, respectively, with the top-associated SNP. SNP names shown are as in dbSNP130 where available. Otherwise, the Illumina Immunochip manifest name is shown. For example, 1kg_17_29697448 indicates the location of SNP on chromosome 17 at position 29697448 (hg18).

**Figure 3 | SNPs of type I IFN-responsive genes are associated with *Candida*-induced cytokine levels.** Correlation plots showing the association between genotypes in the x axis and cytokines levels in the y axis. Association between (a) TNF-α levels and genotypes at *IRF1* SNP rs2548997, (b) IL-10 levels and genotypes at *IRF1* SNP rs1124211, (c) TNF-α and genotype at STAT1 SNP rs2280234 and (d) IL-6 levels and genotypes at STAT1 SNP rs41511150.
IRF3, IRF9 and IRF5, IRF7 expression in CMC patients compared with healthy controls, were measured in cell culture supernatants using enzyme-linked immunosorbent assay (ELISA). (c) A total of 5 x 10^5 PBMC in a volume of 200 μl were stimulated for 2 and 7 days, with heat-killed C. albicans (1 x 10^6 ml⁻¹), in the absence or presence of IFN-γ, IL-17 and TNF-α were measured in cell culture supernatants using ELISA. After stimulation, supernatants were pooled and stored at −20 °C until further assayed. Data were analysed using the Wilcoxon-signed rank test. Data are presented as mean ± s.e.m.

To determine the role of these 27 genes we calculated Gene ontology enrichment scores using the Database for Annotation, Visualization and Integrated Discovery. Enriched gene clusters were generated using Functional Annotation Clustering. Gene ontology terms associated with response to type I IFN and viral infections showed significant enrichment (P = 1.09 x 10⁻14) (Supplementary Table S4).

**Discussion**

The last decade has witnessed a significant increase in knowledge related to the mechanisms involved in antifungal host defence. However, most of this knowledge is based on in vitro and animal studies; these studies led to hypotheses to be tested in human systems, leading to a somewhat biased view of human immunity against fungi. In the present study we have used a different approach based exclusively on complementary human studies: transcriptomics analysis in human primary cells, correlation of genetic variability and antifungal immunity in healthy volunteers (functional genomics), case–control association studies in patients with disseminated C. albicans infections and assessment of antifungal immunity in CMC patients.

In our initial approach, we designed a hypotheses-generating set of experiments by assessing the transcriptional profile induced in circulating primary leukocytes by C. albicans. Human primary PBMCs were stimulated either with C. albicans or with other inflammatory stimuli. Pathway analysis of the genes induced by Candida indicated two broad categories: (i) a first set of genes that was strongly induced and shared by both C. albicans and other inflammatory stimuli (LPS, MTB or B. burgdorferi), constituted expected pathways involved in inflammation and innate immunity, many of which have important roles in antifungal immunity^18, and (ii) a feature set comprising an expression signature of 101 transcripts (95 genes) that distinguishes the Candida-specific response and is characterized by a strikingly strong and unanticipated type I IFN response.

**Figure 4 | Type I IFNs skew cytokine production from a Th1 towards a Th17 response.** (a) 5 x 10⁵ PBMC in a volume of 200 μl were stimulated for 7 days, in the presence of 10% human pooled serum, with live C. albicans (1 x 10⁶ ml⁻¹), in duplo. IFN-α and IFN-β were measured in cell culture supernatants using enzyme-linked immunosorbent assay (ELISA). (b) 5 x 10⁵ PBMC in a volume of 200 μl were stimulated for 1 and 2 days, with heat-killed C. albicans (1 x 10⁶ ml⁻¹), in the absence or presence of anti-IFN-α/βR2, in duplo. IFN-γ, IL-1β and TNF-α were measured in cell culture supernatants using ELISA.

(c) A total of 5 x 10⁵ PBMC in a volume of 200 μl were stimulated for 2 and 7 days, with heat-killed C. albicans (1 x 10⁶ ml⁻¹), in the absence or presence of IFN-α or IFN-β, in duplo. When cells were cultured for 7 days, this was done in the presence of 10% human-pooled serum. IFN-γ, IL-17 and IL-22 were measured in cell culture supernatants using ELISA. (a–c) After stimulation, supernatants were pooled and stored at −20 °C until further assayed. Data were analysed using the Wilcoxon-signed rank test. Data are presented as mean ± s.e.m.
Because the identification of the type I IFN pathway as the most prominent Candida-specific transcriptional signature was surprising, we initiated a series of studies to confirm this finding. First, we assessed whether genetic polymorphisms in several of the genes identified in the Candida-specific data set influenced susceptibility to systemic Candida infections. In a cohort of patients with candidemia that were genotyped using the Immunochip platform (Illumina), on which 11 genes of the Candida fungal infections. In a cohort of patients with candidemia that were genotyped using the Immunochip platform (Illumina), on which 11 genes of the 11 type I IFN pathway-induced genes was defective in CMC patients harbouring a gain-of-function mutation in STAT1 (ref. 15). These mutations lead to increased STAT1 phosphorylation and IFNγ production, while decreasing IL-17 production. Liu et al., previously demonstrated that type I IFNs decrease IL-17 production in vitro and in vivo. TNF-α production did not seem to be influenced by blocking the IFNAR, suggesting that genetic effects in STAT1 exert their effect on TNF-α production either through direct intracellular pathways, or indirectly through IFNγ. Interestingly, C. albicans seems to induce only IFN-β, rather than IFN-α, consistent with earlier studies in mice.

Finally, using both quantitative PCR and RNA sequencing analysis, we demonstrated that the expression of type I IFN pathway-induced genes was defective in CMC patients harbouring a gain-of-function mutation in STAT1 (ref. 15). These mutations lead to increased STAT1 phosphorylation and IFNγ signalling. Liu et al. demonstrated increased STAT1 phosphorylation in response to IFNγ stimulation in U3C cells transfected with mutated STAT1. They hypothesize that IFN-targeted genes can lead to decreased IL-17 production. However, freshly isolated PBMC from CMC patients do not produce any IFNγ in response to C. albicans stimulation. Therefore, it is more likely that the inability of mutated STAT1 to form heterodimers with STAT3 and STAT4 causes defective Th1/
Figure 6 | Heatmap of response of CMC patients and controls to C. albicans in vitro stimulation. In this analysis the standardized fold change gene expression in stimulated versus unstimulated cells was calculated. The scale of this heatmap is given as standardized log2 fold change with a range from -1.8 (blue) to +1.8 (red).
Th17 responses\(^{17}\), which are likely to represent the main immunological defect in this disease. Additional defects in the expression of type I IFN-induced genes, as shown here, may also contribute to the increased susceptibility to mucosal fungal infections. Whether this is truly caused by a defect in STAT1-STAT2 heterodimerization remains to be demonstrated.

Taken together, these complementary studies represent a compelling body of evidence that the type I IFN pathway is an important component of the immune response during infections with \textit{C. albicans} in humans. Moreover, the data presented here are supported by emerging information from murine studies. For example, Bourgeois et al.\(^{22}\) and Biondo et al.\(^{23}\) have reported that mouse conventional DCs produce IFN-\(\beta\) upon stimulation with \textit{Candida} spp. and have proposed a role for MyD88, TLR7 and TLR9 in this induction. On the other hand, these results are relatively surprising, as no earlier study has reported a role for type I IFNs in human antifungal host defence. In this respect, primary immunodeficiencies in type I IFN pathway are mainly associated with viral, rather than fungal, infections\(^{24}\). Therefore, our data indicate that type I IFN defects determine an increase susceptibility risk for fungal infections, as demonstrated here by the association with candidemia in risk groups, rather than an overt immunodeficiency associated with severe fungal infections.

The precise effect of type I IFNs for anti-\textit{Candida} host defence is still being debated. One study in type I IFNAR knockout mice suggested a deleterious role for this pathway\(^{22}\), whereas another study reported that IFNAR--/-- mice die from their inability to control fungal growth\(^\text{23}\). Although type I IFNs have an important role in the immune response against viral infections\(^{25}\), they seem to be deleterious in bacterial infections\(^{26}\). In humans with \textit{Candida} infections, the role of type I IFNs is not yet settled, although the defects seen in type I IFN gene expression in CMC patients, the susceptibility to candidemia in risk groups, rather than an overt immunodeficiency associated with severe fungal infections.

Although viruses have long been known to induce type I IFN production, other microorganisms have been recently reported to induce this pathway. MTB stimulation induces the expression of IFNB and IFNA1 in human macrophages\(^{27}\) and in murine bone marrow-derived macrophages\(^{28}\), type I IFNs are required for caspase-1 activation during \textit{Francisella tularensis} infection\(^{29}\) and \textit{Listeria monocytogenes} induces the transcription of IFNB in macrophages\(^{30}\). However, the complete transcriptional profile of the type I IFN pathway performed here shows important differences from the type I IFN profile induced by MTB\(^{31}\) and is significantly more pronounced for \textit{Candida} than for MTB (Supplementary Fig. S4). Interestingly, the very strong induction of type I IFN pathway genes by \textit{Candida} may explain the well-known, yet unexplained, beneficial effects of injecting \textit{Candida}-derived extracts for the treatment of warts in patients\(^{32,33}\).

In summary, this work presents complementary arguments from several studies to propose that the type I IFN pathway has an important role in the immune responses to the fungal pathogen \textit{C. albicans} in humans. The cross-validation between these approaches supports the value of this systems biology approach, combining transcriptomics with functional genomics and immunological data. Using this approach, we have confirmed the role of pathways known to be involved in antifungal host defence (for example, common inflammatory pathways and the Th1/Th17 pathways) and we have identified a novel pathway (the type I IFN pathway). It is expected that this knowledge will have an important contribution in devising novel immunotherapeutic strategies for fungal infections, as well as influencing decision-making for vaccine design.

**Methods**

**Study population.** For assessing the transcriptome and the cytokine production capacity, blood was collected after written informed consent from healthy volunteers. Adult patients with candidemia were enrolled after informed consent (or waived as approved by the Institutional Review Board) at the Duke University Hospital (DUMC, Durham, North Carolina, USA) and Radboud University Nijmegen Medical Centre (RUMC, Nijmegen, The Netherlands). The study was approved by the Institutional Review Boards at each study centre, and enrolment occurred between January 2003 and January 2009 (ref. 34). The study was performed in accordance with the declaration of Helsinki. In addition, blood was collected from two patients with CMC (on two different occasions) and eight healthy controls to investigate the induction of IFN genes by \textit{Candida}. After informed consent was given, blood was collected by venipuncture into 10 ml EDTA syringes (Monocyte, \(\times\) Hertogenbosch, The Netherlands).

**PBMC isolation.** Separation and stimulation of PBMCs was performed as described previously\(^{35}\). Briefly, the PBMC fraction was obtained by density centrifugation of diluted blood (1 part blood to 1 part pyrogen-free saline) over Ficoll–Paque (Pharmacia Biotech, Uppsala, Sweden). PBMCs were washed twice in saline and suspended in culture medium. The cells were counted in a haemocytometer and their number was adjusted to \(5 \times 10^6\) ml\(^{-1}\).

**Cell stimulation.** For the transcriptome analysis, cells were stimulated for 4 or 24 h with RPME, heat-killed \textit{B. burgdorf}\(^{20}\), \textit{C. albicans} (UC 820) (1 \(\times 10^6\) ml\(^{-1}\)/ml), \textit{E. coli}-derived LPS (1 \(\times 10^6\) ml\(^{-1}\)) or E. coli-derived LPS (1 \(\times 10^6\) ml\(^{-1}\)). To correlate cytokine production to SNPs in the type I IFN pathway, 5 \(\times 10^6\) PBMCs from healthy volunteers were stimulated with heat-killed \textit{C. albicans} (1 \(\times 10^6\) ml\(^{-1}\)) for 24 h. To investigate the effect of type I IFNs on cytokine production, 5 \(\times 10^6\) PBMCs were stimulated with heat-killed \textit{C. albicans} (1 \(\times 10^6\) ml\(^{-1}\)) in the absence or presence of IFN-\(\alpha\) (1 \(\times 10^6\) U ml\(^{-1}\)). To correlate cytokine production to SNPs in the type I IFN pathway, 5 \(\times 10^6\) PBMCs from healthy volunteers were stimulated with heat-killed \textit{C. albicans} (1 \(\times 10^6\) ml\(^{-1}\)) for 24 h. To investigate the effect of type I IFNs on cytokine production, 5 \(\times 10^6\) PBMCs were stimulated with heat-killed \textit{C. albicans} (1 \(\times 10^6\) ml\(^{-1}\)) in the absence or presence of IFN-\(\alpha\) (1 \(\times 10^6\) U ml\(^{-1}\)).

**Transcriptome analysis.** Global gene expression was profiled using Illumina Human HT-12 Expression BeadChip according to manufacturer’s instructions. Image analysis, bead-level processing and quantile normalization of array data were performed using the Illumina LIMS platform, BeadStudio. A gene-wise linear model was fitted to the data and was performed in R programming language (http://www-r-project.org/), utilizing functions from Linear Models for Microarray Data\(^{36}\). For each probe, a moderated \(t\)-statistic (with s.e. moderated across genes) was computed using a Bayesian model. The associated p-value was computed using a Java decision-making for vaccine design.

**Cytokine measurements.** The concentration of IL-1\(\beta\), IL-17, IL-22, TNF-\(\alpha\) (R&D Systems, Abingdon, UK), IL-6, IL-8, IL-10, IFN-\(\gamma\) (Sanquin, Amsterdam, the Netherlands) and IFN-\(\alpha\), IFN-\(\beta\) (PBL Interferon Source, Piscataway, USA) was measured in cell culture supernatants using enzyme-linked immunosorbent assay according to the instructions of the manufacturer.

**SNP selection.** DNA was isolated using the Gentra Puregene Blood kit (Qiagen, Venlo, The Netherlands), according to the protocol of the manufacturer. Illumina Immunochip is a custom-designed high-density array to densely genotype 186 loci associated with 12 different autoimmune or inflammatory diseases, reaching genome-wide association study significance threshold \((P < 5 \times 10^{-8})\)\(^\text{14}\). The candidemia cohort and volunteers DNA samples were genotyped on this Immunochip Illumina platform. Only SNPs in 11 of the 101-gene data set identified earlier were studied, as the other genes were not represented on the Immunochip. We selected tag-SNPs (Supplementary Table S1) at each region that were located within \(\pm 250\) kb of the locus by employing Haploview\(^{44}\) and we excluded SNPs with \(\chi^2\geq 0.8\) from association analysis.
Statistical analysis. Analyses were performed using PLINKv1.07 (ref. 45,46). We performed Multidimensional scaling (MDS) analysis to reveal genetic background of cases and controls in Candidemia cohort (Supplementary Fig. 58). The association between tag-SNPs and Candidemia susceptibility was tested by logistic regression with the first four components from MDS analysis as covariates. The correlation between cytokine levels and genotypes was tested with Wilcoxon rank sum test using R. P < 0.05 was considered significant.

Real-time PCR. Two million freshly isolated PBMCs were incubated with various stimuli as described above. After 24 h of incubation at 37 °C, total RNA was extracted in 1 ml of Dense lysis Reagent (Invitrogen, Breda, The Netherlands). Isolated RNA was reverse-transcribed into complementary DNA using oligo(dT) primers and MMLV reverse transcriptase. PCR was performed using a 7300 Real-Time PCR system (Applied Biosystems, Lennik, Belgium). Primer sequences for human IFN-β reverse transcriptase. PCR was performed using a 7300 Real-Time PCR system (Applied Biosystems, Lennik, Belgium). Primer sequences for human IFN-β

RNA sequencing. RNA sequencing experiments were performed on 4 animals using RNA-seq technology. RNA was extracted from the Agilent 2100 Bioanalyzer. RNA adapters were hybridized and ligated to the fragmented RNA, followed by reverse transcription. After purification and size-selection of RNA using Agencourt AMPure XP Reagent beads, the cDNA was amplified and used as input for the SOLiD system bead preparation using the SOLiD Easy bead system (E120 scale). Paired-end sequencing (25 bases) was performed on one flowcell of a 5500XL (Life Technologies). Enrichment of mRNA was done using the manufacturer's instructions. For measurement of mRNA levels, we used the following oligo sets: 33,544 transcripts were filtered on the Agilent 2100 Bioanalyzer, 9,360 transcripts were considered; 7,064 transcripts were used as a reference gene, for which the primers were: 5′-ATG-AGT-GCA-GGT-TTA-TTA-GG-3′ and antisense: 5′-CCA-AAT-GCC-GCA-TCT-TCA-AAC-3′ (reverse). The PCR conditions were as follows: 2 min at 50 °C and 10 min at 95 °C, followed by 40 cycles of PCR reaction at 95 °C for 15 s, and 60 °C for 1 min.

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Author contributions

Additional information
Accession Codes: Transcriptomics data have been deposited in the Gene Expression Omnibus (GEO) database under accession code GSE42606. RNA sequencing data have been deposited in the GEO database under accession code GSE42630.

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