Eur J Clin Microbiol Infect Dis (2015) 34:963–974
DOI 10.1007/s10096-014-2309-2

ARTICLE

# The RIG-I-like helicase receptor MDA5 (*IFIH1*) is involved in the host defense against *Candida* infections

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Received: 6 November 2014 / Accepted: 22 December 2014 / Published online: 13 January 2015


**Abstract** The induction of host defense against *Candida* species is initiated by recognition of the fungi by pattern recognition receptors and activation of downstream pathways that produce inflammatory mediators essential for infection clearance. In this study, we present complementary evidence based on transcriptome analysis, genetics, and immunological studies in knockout mice and humans that the cytosolic RIG-I-like receptor MDA5 (*IFIH1*) has an important role in the host defense against *C. albicans*. Firstly, *IFIH1* expression in macrophages is specifically induced by invasive *C. albicans* hyphae, and patients suffering from chronic mucocutaneous candidiasis (CMC) express lower levels of MDA5 than healthy controls. Secondly, there is a strong association between missense variants in the *IFIH1* gene (rs1990760 and rs3747517) and susceptibility to systemic *Candida* infections. Thirdly, cells from *Mda5* knockout mice and human peripheral blood mononuclear cells (PBMCs) with different *IFIH1* genotypes display an altered cytokine response to *C. albicans*. These data strongly suggest that MDA5 is involved in immune responses to *Candida* infection. As a receptor for viral RNA, MDA5 until now has been linked to antiviral host defense, but these novel studies show

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**Electronic supplementary material** The online version of this article (doi:10.1007/s10096-014-2309-2) contains supplementary material, which is available to authorized users.

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unexpected effects in antifungal immunity as well. Future studies are warranted to explore the potential of MDA5 as a novel target for immunotherapeutic strategies.

**Keywords** Candida albicans · Host defense · Candidemia · Genetic susceptibility · MDA5 · *IFIH1*

## Introduction

*Candida* species are one of the most common human fungal pathogens. Oropharyngeal and vaginal *Candida* infections are often diagnosed in the population, while systemic candidiasis is the fourth most common form of bloodstream infections in the USA, with mortality rates reaching up to 40 % [1–4]. In addition to the known risk factors (immunosuppressive medication, parenteral nutrition, prolonged intensive care hospitalization, etc.), recent studies have described several genetic risk factors that contribute to the cause and severity of systemic candidiasis [5, 6]. Several monogenic disorders that result in primary immunodeficiencies increase the susceptibility to *Candida* infection, as demonstrated for mutations in *CARD9* and *STAT1* [7–9]. Common genetic variants, for example in pattern recognition receptors (e.g., *Dectin-1* and *TLR1*) and interleukins (e.g., *IL-4*, *IL-10*, and *IL-12B*), also increase the risk of infection by affecting *Candida* recognition and cytokine signaling [6, 10].

However, more insight is needed to identify host defense pathways that are suitable targets for novel immunotherapeutic approaches. Recently, we described that the type I interferon (IFN) pathway plays a central role in host defense against *C. albicans* [11]. In the present work, we demonstrate that MDA5 (*IFIHI*), a RIG-I-like receptor, until now described as a receptor of viral RNA that induces a signaling pathway leading to the production of type I IFNs, is directly involved in the inflammatory response against *Candida* infections in humans. To this end, we present complementary evidence based on transcriptome analysis, genetics, and functional immunological studies in knockout mice and in healthy humans, as well as in patients suffering from systemic candidiasis or chronic mucocutaneous candidiasis (CMC). This is the first time that a receptor of the RIG-I-like helicase family has been shown to be involved in the antifungal immune response.

## Materials and methods

### Transcriptome analysis of *Candida*-stimulated macrophages

CD14+ monocytes derived from healthy volunteers were differentiated into macrophages using macrophage colony-stimulating factor (M-CSF) for 7 days. Macrophages ($2\times10^5$/well) were stimulated for 4 or 24 h with either culture medium, a wild-type *C. albicans* strain (UC820), or an *HGC1* null mutant strain. This mutant is unable to form hyphae and is, therefore, locked in a yeast form, although this form does not correspond to wild-type yeast cells, as the mutant expresses several filament-specific genes [12]. As yeast–hyphal transition is considered an invasive trait of *C. albicans*, gene expression induced by either the wild-type (hyphae) or *HGC1* null (yeast) strains was profiled and compared to unstimulated macrophages, as described previously [11]. We identified genes that showed significant differential expression in at least one of the conditions after Benjamini–Hochberg correction ($p<0.05$ and >2-fold change in expression). From this set, we selected the 62 genes that are exclusively induced after stimulation with wild-type *C. albicans* for 24 h.

### Candidemia and control cohorts

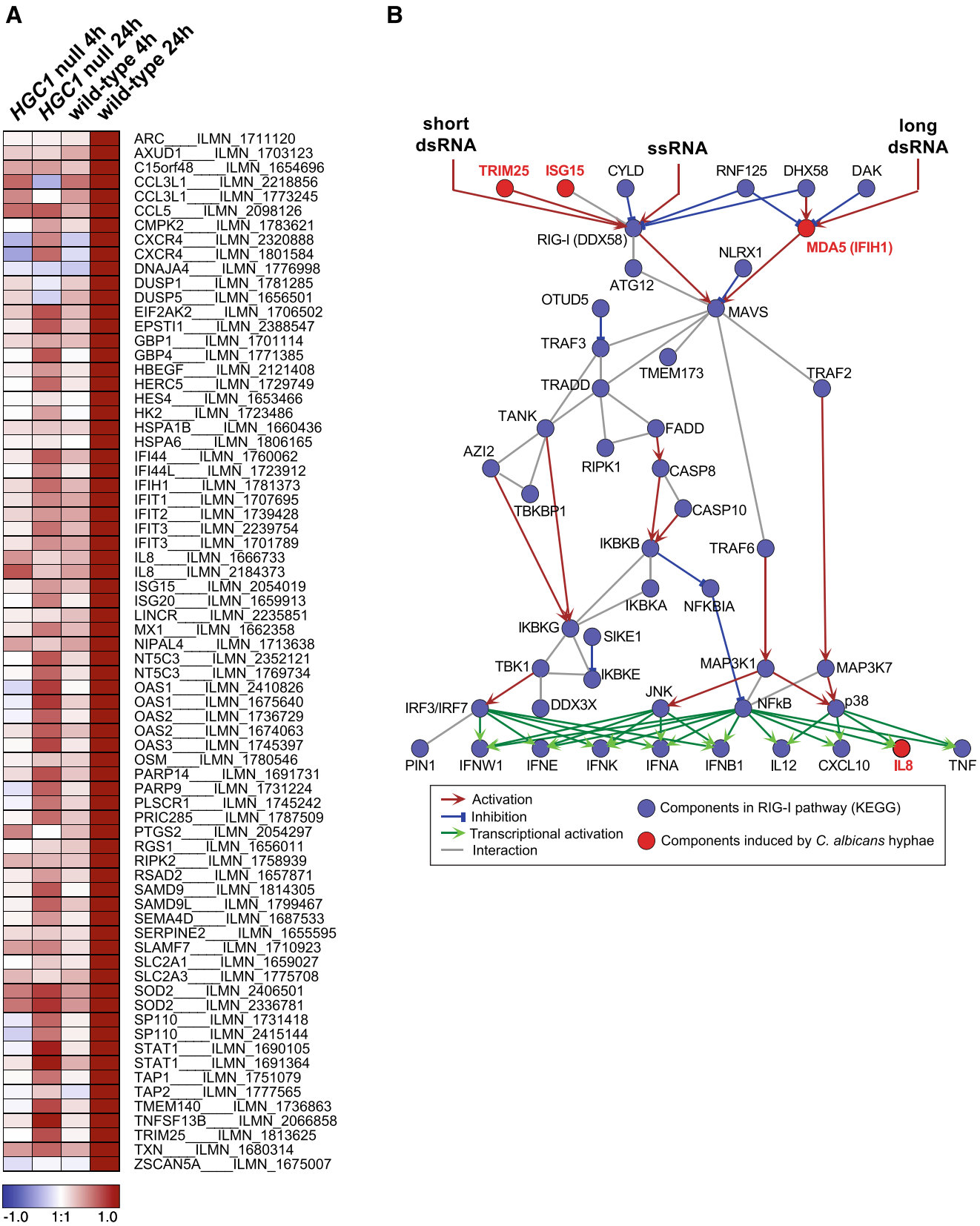
In this study, we included 227 unrelated adult Caucasian candidemia patients (described in detail in [6]). Patient enrollment took place after confirmation of at least one positive blood culture for a *Candida* species. The control cohort of 176 Caucasians consists of non-infected (candidiasis-free) matched patients from the same medical centers as the patient cohort. Controls were recruited consecutively from the same hospital wards as infected patients during the study period, with a similar balance of medical, surgical, and oncology patients in the case and control groups. Review boards of the involved medical centers approved the study and patients were enrolled after giving written informed consent (see Supplementary Methods).

### Genotyping and genetic analyses

Cases and controls in the candidemia cohort were genotyped on the Illumina Immunochip single nucleotide polymorphism (SNP) array platform, which contains ~200,000 SNPs focused

**Fig. 1** Transcriptional changes in macrophages stimulated with *Candida albicans*. **a** The heat map shows differential gene expression after 4 or 24 h of stimulation of human macrophages with yeast-locked *HGC1* null *C. albicans* (which are unable to form hyphae, but are known to express several hyphal proteins) or wild-type invasive *C. albicans* (that can form hyphae) compared to expression levels in unstimulated macrophages (control). Sixty-two genes exhibited a significant change in expression level (Benjamini–Hochberg-corrected $p<0.05$ and>2fold change in expression) specifically after 24 h of stimulation with wild-type *Candida*, during which germination into hyphae takes place. The signal-to-noise ratio scaled to the maximum absolute deviation is shown for each probe corresponding to the 62 differentially expressed genes. **b** *C. albicans* hyphae-induced genes, *IFIH1*, *TRIM25*, *ISG15*, and *IL8* (indicated in *red*), are components of the RIG-I-like receptor (RLR) signaling pathway. These genes represent both the MDA5 (*IFIH1*) and RIG-I (*ISG15* and *TRIM25*) branches, as well as inflammatory cytokines that are produced by activation of the pathway (*IL8*). Figure based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) map of the RLR pathway [16]

on genomic regions known to be involved in immune-mediated diseases [13]. After the application of quality control filters, we tested 843 SNPs in the *FAP-IFIH1-GCA-KCNH7* linkage disequilibrium (LD) region for candidemia case–control



association. This 405-kb LD region (hg18 coordinates: chr2 162,720–163,125 kb) was defined based on the LD patterns in the larger genomic region (Figure S1). See Supplementary Methods for detailed information.

### Expression analysis of PBMCs

To assess the expression levels of genes in the *FAP-IFIH1-GCA-KCNH7* LD region, blood was collected from healthy volunteers. Peripheral blood mononuclear cells (PBMCs, $5\times10^5$/well) were stimulated with either culture medium, $1\times10^6$/ml heat-killed *Borrelia burgdorferi* [14], $1\times10^6$/ml heat-killed *Candida albicans* (UC820) [15], 10 ng/ml *Escherichia coli*-derived lipopolysaccharide (LPS), or $1\times10^7$/ml sonicated *Mycobacterium tuberculosis* (MTB) (Hv37Rv) for either 4 or 24 h. Gene expression was profiled using the Illumina HumanHT-12 Expression BeadChip kit [11]. Additionally, gene expression was assessed in PBMCs from two patients suffering from CMC due to *STAT1* mutations (Arg274Trp) [8]. Cells were stimulated with *C. albicans* for 4 h and gene expression was measured by RNA sequencing as described elsewhere [11].

### PBMC stimulations experiments

$5\times10^5$ isolated PBMCs per well were stimulated with either heat-killed *C. albicans* yeast or hyphae (UC820, $1\times10^6$/ml) for 24 h (IL-10) or 7 days (IL-17 and IFN-γ). Additionally, PBMCs were stimulated with MTB (1 μg/μl) for 24 h. Supernatants were collected and measured for IL-10 and IFN-γ (Sanquin, Amsterdam, The Netherlands) and IL-17 cytokines (R&D Systems, Abingdon, UK). We excluded samples that showed a positive RPMI (control condition with contamination) or errors in the experimental readout.

### *Mda5* knockout mice studies

*Mda5*$^{-/-}$ mice on C57BL/6 J background have been backcrossed at least ten times and were kindly provided by Dr. M. Colonna. Splenocytes were isolated from wild-type and *Mda5*$^{-/-}$ mice and stimulated with RPMI, Poly I:C, and heat-killed *C. albicans* yeasts or hyphae for 24 h. Cytokines were measured in supernatants by enzyme-linked immunosorbent assay (ELISA). For quantitative real-time polymerase chain reaction (qPCR) experiments, splenocytes from both *Mda5* knockout mice and B6 control mice were stimulated for 24 h with heat-killed *C. albicans* hyphae. RNA was isolated according to the TRIzol® isolation protocol (Life Technologies).

## Results

### *Candida* germination induces expression of RLR pathway components in macrophages

*C. albicans* is a dimorphic fungus that exists either in a colonizing yeast form or as an invasive filamentous form (hyphae). To identify the specific transcription profile induced by fungal germination into hyphae, we profiled the transcriptome of macrophages stimulated with either wild-type (which develop hyphae) and *HGC1* null strains of *C. albicans* (which have a yeast-locked phenotype, though are different from wild-type) [12]. Sixty-two genes exhibited significant differential expression specifically in macrophages stimulated with *Candida* hyphae for 24 h (required for hyphal formation), but not for 4 h (Benjamini–Hochberg-corrected $p<0.05$ and >2-fold change in expression compared to unstimulated macrophages, Fig. 1a). Many of these genes are involved in IFN signaling, consistent with a previous study [11]. Interestingly, four of the genes induced by *Candida* hyphae stimulation (*IFIH1*,

**Table 1** Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment for genes that are specifically induced in macrophages stimulated with wild-type *Candida* for 24 h

| KEGG identifier | Pathway | *p*-Value | Fold enrichment | Genes |
|---|---|---|---|---|
| hsa04622 | RIG-I-like receptor signaling pathway | $4.3\times10^{-3}$ | 11.5 | IFIH1, ISG15, IL8, TRIM25 |
| hsa04060 | Cytokine–cytokine receptor interaction | $6.6\times10^{-3}$ | 4.7 | OSM, TNFSF13B, IL8, CXCR4, CCL3L1, CCL5 |
| hsa04612 | Antigen processing and presentation | $6.6\times10^{-3}$ | 9.8 | TAP2, TAP1, HSPA6, HSPA1B |
| hsa04062 | Chemokine signaling pathway | $1.1\times10^{-2}$ | 5.4 | IL8, CXCR4, CCL3L1, CCL5, STAT1 |
| hsa04621 | NOD-like receptor signaling pathway | $3.4\times10^{-2}$ | 9.8 | IL8, RIPK2, CCL5 |
| hsa05120 | Epithelial cell signaling in *Helicobacter pylori* infection | $4.0\times10^{-2}$ | 9.0 | IL8, HBEGF, CCL5 |

Enrichment for KEGG pathway components [16] was determined using the functional annotation tool of the DAVID suite [17]. Background: all human genes

*ISG15*, *IL8*, and *TRIM25*) are components of the RIG-I-like receptor (RLR) signaling pathway, significantly more than expected for a random set of genes ($p=4.3\times10^{-3}$, 11.5-fold enrichment, Table 1).

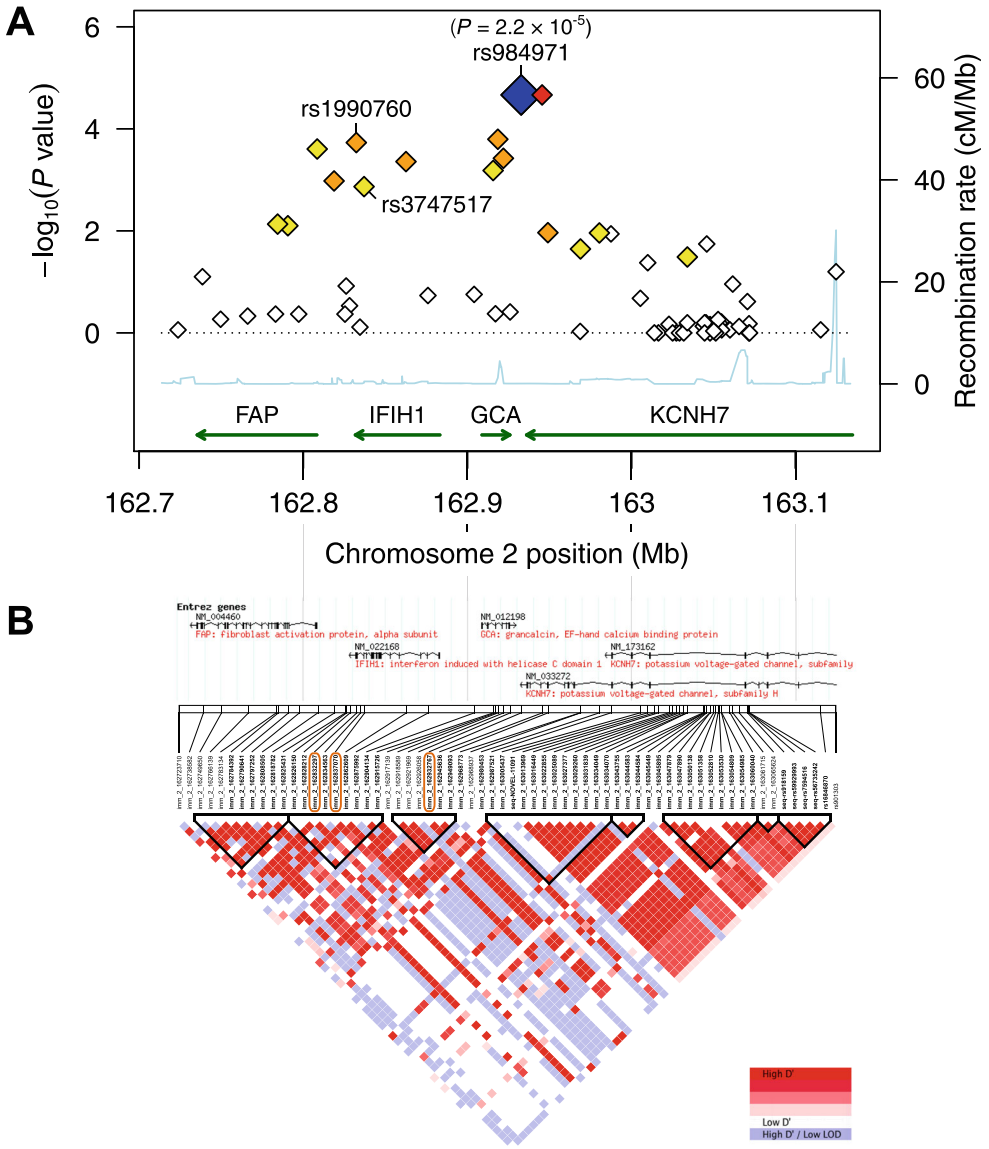



**Fig. 2** **a** Regional association plot and **b** linkage disequilibrium (LD) map for the *FAP-IFIH1-GCA-KCNH7* LD region on chromosome 2. **a** Sixty-four single nucleotide polymorphisms (SNPs) with minor allele frequency (MAF)>5 % in 403 Caucasian individuals of the candidemia cohort (cases and controls together) were assessed for genotypic association with candidemia. The resulting $-\log_{10}$(genotypic *p* values) (left *y*-axis) are plotted as a function of genomic coordinates (hg18, *x*-axis). The *blue diamond* highlights the most significant SNP, along with its *p*-value (rs984971). rs1990760 and rs3747517 are the only two significant missense SNPs; both are in the coding region of *IFIH1*. Recombination rates, estimated from the CEU, YRI, and JPT+CHB HapMap populations (HapMap 2, Release 22) [19], are plotted to reflect the local LD structure (right *y*-axis, *cyan line*). SNPs are colored according to the degree of LD with the most significant SNP, rs984971 (R-squared, calculated across the controls in the candidemia cohort; from strong to weak LD: *red*: r2≥0.8; *orange*: 0.5≤r2<0.8; *yellow*: 0.2≤r2<0.5; *white*: r2<0.2). Genes with their direction of transcription are shown at the bottom; *KCNH7* is only partly in this region. **b** LD patterns across the 405-kb *FAP-IFIH1-GCA-KCNH7* LD region are calculated based on genotypes of control individuals in the candidemia cohort, measured using the Immunochip SNP array. The intersections of the diagonals between pairs of SNPs are colored according to the degree of LD, which is calculated as D' and LOD: SNPs with D' values between 0 and 1 and with LOD≥2 are colored from white to red. Haplotype blocks (*triangles with bold black borders*) are regions where at least 95 % of SNPs are in strong LD, defined by high D' values [20]. Chromosome 2 coordinates (hg18) and Entrez genes are shown at the top. The *orange boxes* around SNP identifiers indicate the top SNP and two *IFIH1* missense SNPs significantly associated with susceptibility to candidemia (see Table 2). The corresponding R-squared LD map for the candidemia cohort is depicted in Figure S1d. See Figures S1a–c for R-squared and D'/LOD LD maps calculated based on the HapMap CEU population

**Table 2** Selection of single nucleotide polymorphisms (SNPs) in the *FAP-IFIH1-GCA-KCNH7* linkage disequilibrium (LD) region that are significantly associated with susceptibility to candidemia

| SNP | Immunochip | Closest gene(s) | Alleles (dbSNP) | Functional class (AA change) | BH-corrected genotypic *p*-value |
|---|---|---|---|---|---|
| rs984971 | imm_2_162932767 | *GCA* \| *KCNH7* | A/G | Intergenic | $6.9\times10^{-4}$ |
| Genotypes | GG | GA | AA | | |
| Controls | 25 (14.2 %) | 103 (58.5 %) | 48 (27.3 %) | $p=2.2\times10^{-5}$ | |
| Cases | 25 (11.0 %) | 89 (39.2 %) | 113 (49.8 %) | | |
| Alleles | G | A* | | | |
| Controls | 153 (43.5 %) | 199 (56.5 %) | $p=2.2\times10^{-4}$ | | |
| Cases | 139 (30.6 %) | 315 (69.4 %) | OR, G vs. A=0.57 (0.43–0.77) | | |
| rs1990760 | imm_2_162832297 | *IFIH1* | C/T | Missense (Ala946Thr) | $3.0\times10^{-3}$ |
| Genotypes | CC | CT | TT | | |
| Controls | 31 (17.6 %) | 99 (56.3 %) | 46 (26.1 %) | $p=1.9\times10^{-4}$ | |
| Cases | 37 (16.3 %) | 87 (38.3 %) | 103 (45.4 %) | | |
| Alleles | C | T* | | | |
| Controls | 161 (45.7 %) | 191 (54.3 %) | $p=3.7\times10^{-3}$ | | |
| Cases | 161 (35.5 %) | 293 (64.5 %) | OR, C vs. T=0.65 (0.49–0.87) | | |
| rs3747517 | imm_2_162837070 | *IFIH1* | T/C (A/G) | Missense (His843Arg) | $8.7\times10^{-3}$ |
| Genotypes | TT | TC | CC | | |
| Controls | 12 (6.8 %) | 88 (50.0 %) | 76 (43.2 %) | $p=1.4\times10^{-3}$ | |
| Cases | 20 (8.8 %) | 73 (32.2 %) | 134 (59.0 %) | | |
| Alleles | T | C* | | | |
| Controls | 112 (31.8 %) | 240 (68.2 %) | $p=3.3\times10^{-2}$ | | |
| Cases | 113 (24.9 %) | 341 (75.1 %) | OR, T vs. C=0.71 (0.52–0.97) | | |

Genotypic and allelic associations were assessed using the Fisher's exact test. *p*-Values are shown next to the corresponding contingency tables. Odds ratios (OR, with 95 % confidence intervals) are reported for the allelic association tests and represent the odds of disease for individuals carrying the non-risk allele versus the risk allele. Risk alleles are denoted by an asterisk. BH-corrected genotypic *p*-value: Benjamini–Hochberg correction of the genotypic association test *p*-values for testing multiple SNPs (64 in total). Immunochip: the identifier of the SNP on the Immunochip SNP array. Alleles: the alleles measured with the Immunochip, and the complementary alleles reported by dbSNP (build 138), if they are different. The table shows the top SNP in the LD region, along with the only two significant missense SNPs. All SNPs tested are in Hardy–Weinberg equilibrium in the controls ($p>1\times10^{-3}$). See Table S1 for the full list of significant SNPs

RIG-I-like receptors are well-known intracellular receptors of viral RNA, leading to the production of type I IFNs and proinflammatory cytokines [18]. *IFIH1*, with its protein product known as MDA5, is the receptor of one branch of the RLR pathway (Fig. 1b). *ISG15* and *TRIM25* are involved in the RIG-I branch. Thus, the invasive form of *Candida* induces the expression of components of two branches of the virus-recognition RLR pathway in macrophages.

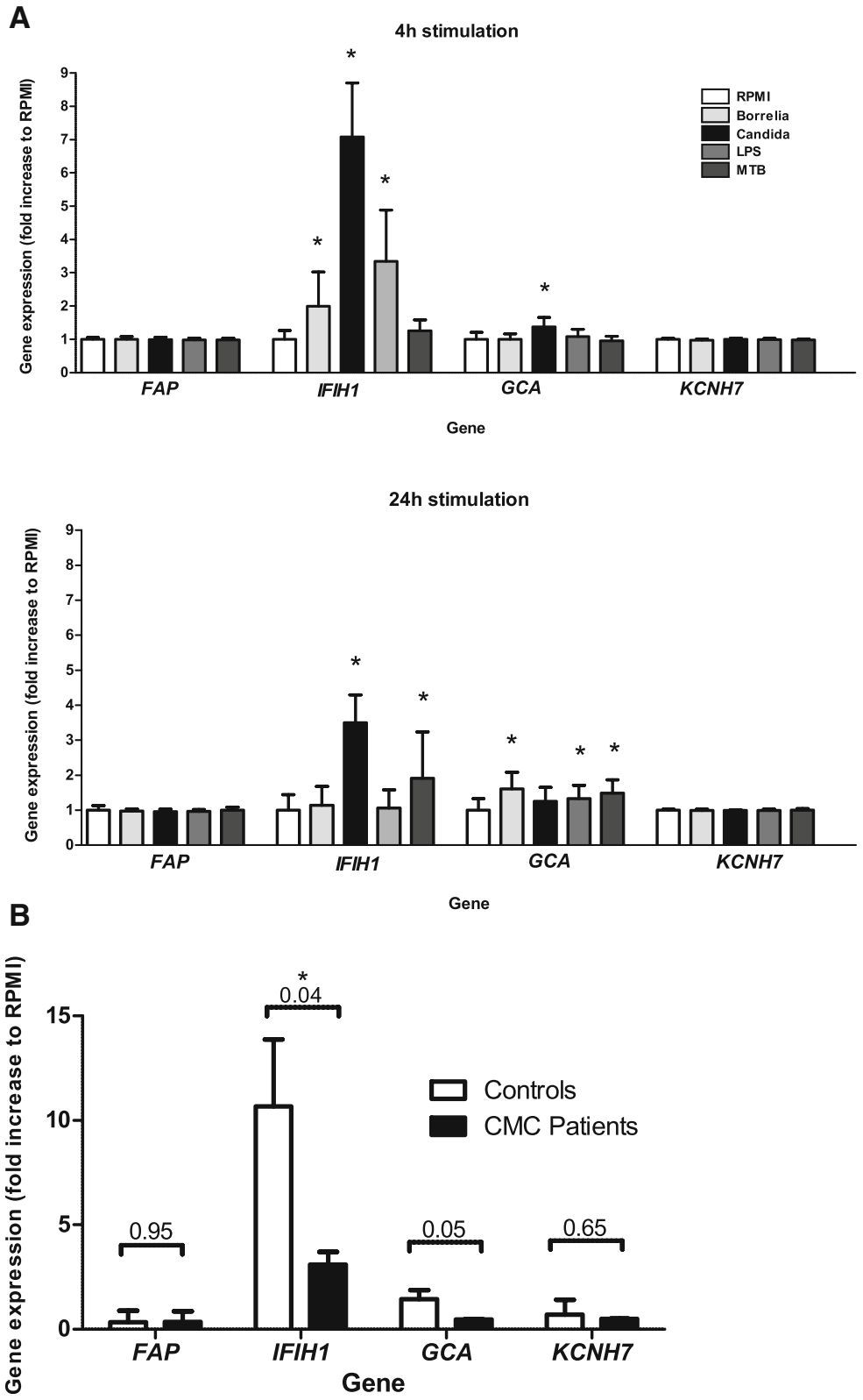
## Genetic variation linked to *IFIH1* modulates susceptibility to candidemia

To validate a role for components of the RLR pathway in invasive *Candida* infection, we investigated whether genetic variation linked to *IFIH1*, *ISG15*, *IL8*, or *TRIM25* correlates with susceptibility to candidemia in patients. Analysis of 64 SNPs associated with *IFIH1* revealed strong associations (Fig. 2a and Table 2). The *IFIH1* locus is present in a 405-kb region on chromosome 2 with low recombination rates (Fig. 2a) and accompanying strong linkage disequilibrium (LD) in both the candidemia cohort and the HapMap CEU population (Figs. 2b and S1) [21–23]. Besides *IFIH1*, the LD region contains the genes *FAP*, *GCA*, and part of *KCNH7*. Fifteen of the 64 SNPs in the *FAP-IFIH1-GCA-KCNH7* LD region differ significantly between cases and controls

**Fig. 3** Transcriptional response of genes in the *FAP-IFIH1-GCA-KCNH7* LD region to various microbial stimuli. **a** Peripheral blood mononuclear cells (PBMCs) from healthy volunteers (minimum $n=23$) were stimulated for either 4 or 24 h with *Borrelia burgdorferi*, *Candida albicans*, *Escherichia coli*-derived lipopolysaccharide (LPS), or *Mycobacterium tuberculosis* (MTB). Gene expression [mean ± standard deviation (SD)] was measured using microarrays and normalized to the control RPMI condition (untreated). *p*-Values (Welch-corrected *t*-test) compared expression distributions of individual stimuli to their respective untreated controls and were Bonferroni-corrected for testing 32 hypotheses (four stimuli across four genes at two time points). The *asterisks* represent all significant comparisons at $\alpha<0.05$. **b** Gene expression (mean ± SD) in PBMCs of healthy controls ($n=3$) and patients suffering from chronic mucocutaneous candidiasis (CMC) ($n=2$) were stimulated with *C. albicans* for 4 h. *p*-Values were calculated using the Welch-corrected *t*-test

(Benjamini–Hochberg-corrected genotypic $p<0.05$, Table S1). The significant SNPs are distributed mainly across the central part of the LD region (Fig. 2a) and the association does not extend beyond the LD region (Figure S2).

An intergenic SNP between *GCA* and *KCNH7* shows the strongest association with candidemia (rs984971, genotypic $p=2.2\times10^{-5}$, allelic $p=2.2\times10^{-4}$, odds of disease 0.43–0.77, Table 2). Although the Immunochip covers missense coding



variants in all four genes, only *IFIH1* harbors significant missense SNPs (rs1990760–Ala946Thr and rs3747517–His843Arg, which are also in strong LD with each other; HapMap CEU: D'=1, r2=0.42; candidemia cohort: D'=1, r2=0.55). Furthermore, rs1990760 and rs3747517 are quantitative trait loci (QTLs) to *IFIH1* expression, with the candidemia risk alleles correlating with higher *IFIH1* expression in PBMCs [22, 24]. These results suggest that *IFIH1* is involved in candidemia.

### *IFIH1* is strongly upregulated upon *Candida* stimulation of PBMCs, while *FAP*, *GCA*, and *KCNH7* are not

To provide additional evidence regarding which genes in the *FAP-IFIH1-GCA-KCNH7* LD region are important for the host response to *Candida*, we assessed gene expression in PBMCs from healthy volunteers after stimulation with various microbes. Stimulation with *C. albicans* resulted in a strong increase of *IFIH1* expression ($p=1.5\times10^{-15}$ at 4 h and $p=1.9\times10^{-12}$ at 24 h, Welch-corrected *t*-tests, Fig. 3a). Of the other LD region genes, only *GCA* was also weakly induced by *C. albicans*. In addition, we compared the expression patterns of *IFIH1* in healthy individuals with two patients suffering from CMC due to a deleterious *STAT1* mutation [8]. CMC patient cells expressed significantly lower levels of *IFIH1* after stimulation with *C. albicans* than cells from healthy individuals ($p=0.04$, Welch-corrected *t*-test, Fig. 3b), while of the other genes, only *GCA* also displayed minor differences ($p=0.05$). Together, the observations in healthy individuals and CMC patients indicate that expression of *IFIH1*, and not expression of the other genes in the LD region, is specifically induced by stimulation with *C. albicans*.

### Genetic variants in *IFIH1* are associated with an altered cytokine profile in response to *Candida*

To investigate the functional consequences of genetic variants associated with *IFIH1* that predispose individuals to candidemia (Table 2), we correlated the genotypes of the SNPs with in vitro cytokines levels upon *Candida* stimulation. A trend was observed towards an increased capacity to release the proinflammatory cytokines IFN-$\gamma$ and IL-17 in cells isolated from individuals homozygous for the risk allele for both *IFIH1* missense polymorphisms (TT for rs1990760; CC for rs3747517) (Fig. 4). In contrast, levels of the anti-inflammatory IL-10 tended to be lower in individuals carrying the risk allele. The top intergenic SNP associated with candidemia (rs984971) did not reveal the same trends (Figure S3). Furthermore, stimulation with other microbial stimuli did not reveal clear correlations between cytokine levels and *IFIH1* missense SNP genotypes (Figure S4), suggesting specificity for *Candida*. Thus, genetic variation in *IFIH1* may influence anti-*Candida* cytokine profiles in vitro.

### Missense SNPs could affect MDA5 protein function

We next sought to gain insight into the possible consequences of having alternative alleles at the *IFIH1* missense SNPs on MDA5 protein function. In silico analysis shows that residue 946 (rs1990760, Ala946Thr) is part of an intrinsically disordered loop [25]. The equivalent loop is rigid in RIG-I, and this differential flexibility contributes to the different RNA binding preferences between MDA5 and RIG-I [26]. The human MDA5 crystal structure has an arginine at position 843 (rs3747517, His843Arg), which interacts with the negatively charged RNA backbone (Figure S5). Histidine would weaken this electrostatic interaction because it is less often positively charged at physiological pH than arginine. Furthermore, position 843 is close to the interface likely involved in interactions between MDA5 monomers (Figure S5) [26]. The formation of MDA5 filaments along the RNA is critical for downstream activation of MAVS [27] and mutation of nearby residues 841 and 842 disrupts signaling [26]. Thus, the Ala946Thr and His843Arg substitutions could alter dsRNA binding selectivity and affinity, and the latter might also affect signaling activity.

### *Mda5* knockout mice have reduced cytokine production in response to *C. albicans*

To provide an additional argument for the role of MDA5 in the anti-*Candida* response, we stimulated splenocytes from *Mda5* knockout and B6 control mice with *C. albicans* yeasts or hyphae. *Mda5*-deficient cells showed a defective production of IFN-β induced by *Candida* (Fig. 5). Similarly, the IL-6 and IL-10 cytokine responses were lower in cells from *Mda5* knockout mice. These differences were more pronounced in stimulations with hyphae compared to the yeast form (Figure S6).

## Discussion

In the present study, we propose that the pattern recognition receptor MDA5, which belongs to the RLR family and plays an important role in antiviral immunity by recognizing viral RNA [18], is also involved in antifungal host defense. MDA5 modulates cytokine production induced in human leukocytes by *C. albicans*, while genetic variants in the *IFIH1* gene that encodes MDA5 influence susceptibility to disseminated candidiasis. Based on these data and the known role of MDA5 in IFN production, it is most likely that this effect is mediated through the induction of type I IFNs.

*C. albicans* is a dimorphic fungus, and germination from yeasts to hyphae is a central process for the invasion of tissues. Surprisingly, transcriptome analysis aiming to identify the

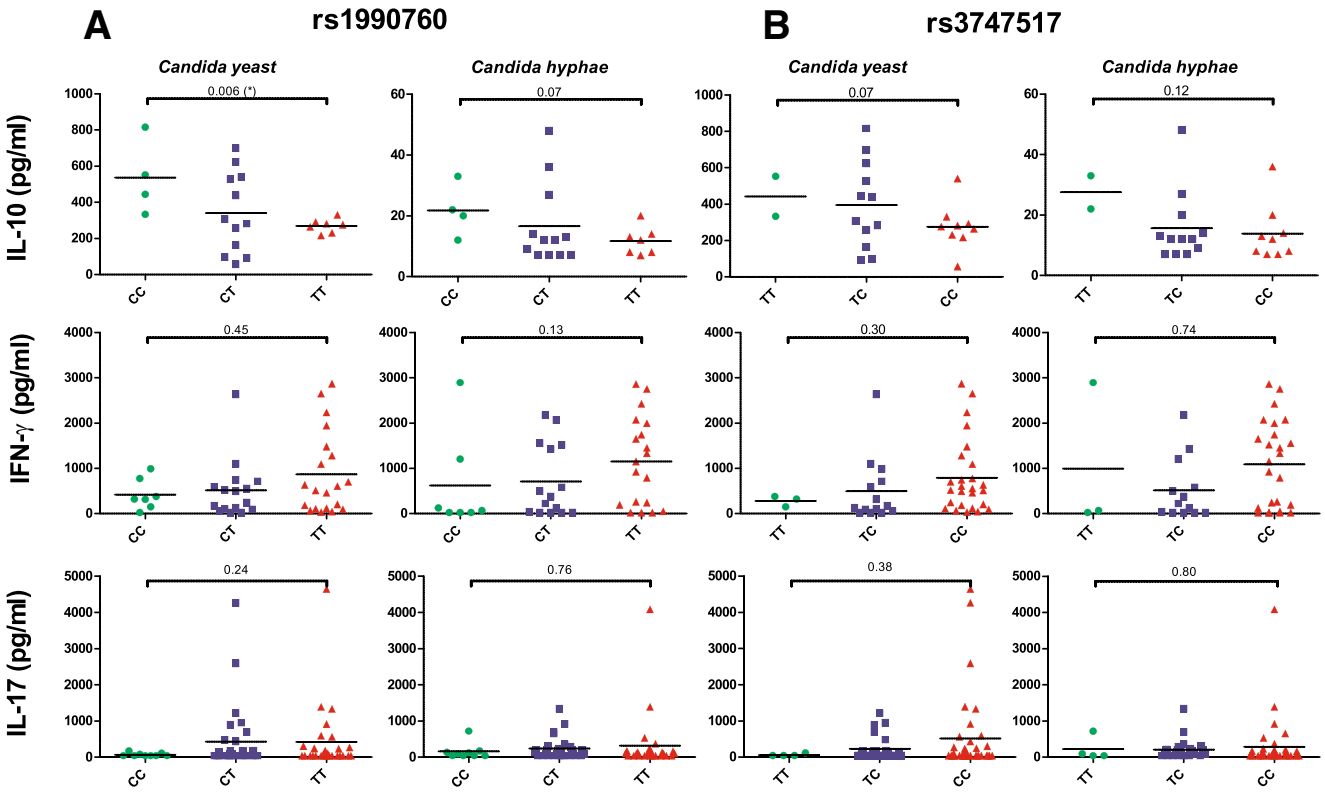



**Fig. 4** *IFIH1* missense SNP genotypes correlate with *Candida*-induced cytokine levels. PBMCs from healthy volunteers with different genotypes for **a** rs1990760 (candidemia risk allele T) and **b** rs3747517 (candidemia risk allele C) were stimulated in vitro with either *C. albicans* yeast or hyphae. Cytokine levels (scatterplots with mean indicated) were measured after 24 h (IL-10) or 7 days (IL-17 and IFN-γ) by enzyme-linked immunosorbent assay (ELISA). *p*-Values were calculated using the Mann–Whitney *U*-test comparing cytokine levels of the two homozygous genotypes

immunological programs induced in human macrophages specifically by *Candida* germination into hyphae identified the MDA5/RIG-I signaling pathway as one of the top targets. The hypothesis that MDA5 is important for host defense against *Candida* was strengthened by the observation that MDA5 induction is defective in cells isolated from patients suffering from CMC. Furthermore, assessment of genetic variation predisposing to candidemia in a patient cohort revealed a strong association between the disease and the genomic region that contains *IFIH1*. While genetic variation in *IFIH1* has previously been shown to influence susceptibility to several autoimmune diseases such as type I diabetes, Graves' disease, and multiple sclerosis [22–24, 28–33], this is the first report of polymorphisms in *IFIH1* linked to a fungal infection. These data are in line with recent studies showing that polymorphisms in other pattern recognition receptors, such as *TLRs* [6, 34–36], or components of the IFN pathway, such as *STAT1* or *IRF1* [11], also influence susceptibility to systemic fungal infections.

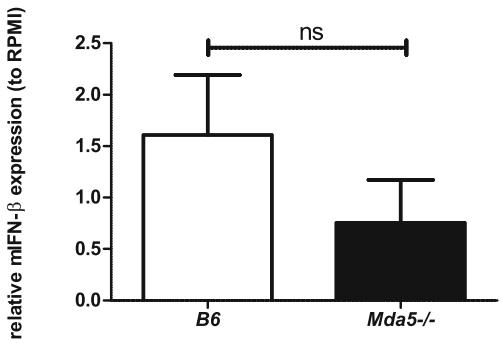



**Fig. 5** Relative gene expression [mean ± standard error of the mean (SEM)] of mouse interferon β (mIFN-β) in splenocytes isolated from B6 control mice (C57BL/6J) and *Mda5* knockout mice, upon stimulation with *C. albicans* hyphae ($10^6$/ml) ($n$=5/group). The *p*-value was not significant at $\alpha<0.05$ (calculated using the Welch-corrected *t*-test)

It is important to point out that the candidemia-associated LD region contains several genes: *FAP* (fibroblast activation protein), *IFIH1* (interferon induced with helicase C domain 1), *GCA* (grancalcin), and *KCNH7* (potassium voltage-gated channel subfamily H member 7). *IFIH1* and grancalcin were the strongest candidates for causing the susceptibility to candidemia, as these genes have known functions in immunity. Grancalcin is abundant in macrophages and neutrophils [37], and is thought to mediate leukocyte adhesion and migration [38]. Gene expression analysis confirmed that *IFIH1* was strongly induced in PBMCs stimulated with *Candida* and *GCA* to lesser extent, while the other genes did not show any expression changes. *IFIH1* and *GCA* are divergently

transcribed neighboring genes with ~25 kb separating their transcription start sites. As such neighboring genes tend to be co-expressed [39, 40], the moderate upregulation of *GCA* in response to *Candida* stimulation could be a by-effect of the strong induction of *IFIH1*, although the genes are still relatively far apart. Importantly, grancalcin-deficient ($Gca^{-/-}$) mice are not more susceptible to candidiasis than wild-type mice [41], which strongly argues against an important role for *GCA* in the immune response against *C. albicans*. Therefore, we concluded that genetic variants influencing *IFIH1* are the most likely cause of the association of the *FAP-IFIH1-GCA-KCNH7* LD region with candidemia.

The candidemia risk alleles of the *IFIH1*-linked SNPs identified in our study have previously been shown to lead to higher expression of *IFIH1* in PBMCs [22, 24]. Furthermore, our protein structure analysis indicates a possibly stronger RNA binding by MDA5 through Arg843, which is encoded by the risk allele C of rs3747517. To gain insight into the downstream immunological effects of *IFIH1* variants, we measured cytokine levels produced by PBMCs with different genotypes for the two *IFIH1* missense SNPs (rs1990760 and rs3747517). These data indicate that cells from individuals bearing the candidemia risk alleles produce more proinflammatory cytokines (IFN-γ and IL-17) and less anti-inflammatory IL-10 in response to *C. albicans* yeast and hyphal forms than cells bearing the protective alleles.

These observations bring into discussion the nature of the involvement of MDA5 in the host defense against *Candida*. MDA5 activates the RLR pathway, leading to the production of type I IFNs during viral infections [42]. A similar biological activity during *Candida* stimulation was shown by our data from *Mda5* knockout mouse splenocytes, which displayed a decreased capacity to induce IFN-β. A role for type I IFNs in antifungal immunity has been recently proposed [11], and MDA5 is likely the receptor that is at least partially responsible for the type I IFN induction during *C. albicans* infection.

Mutations leading to inherently increased expression or activity of MDA5 are likely to increase IFN production [43]. Aberrant production of type I IFNs, in turn, can cause imbalances in the immune response that are reflected in our observed alterations in the levels of other cytokines. The apparent deleterious effect of MDA5 hyperactivity on the anti-*Candida* host defense is consistent with observations that type I IFNs could be harmful for this response: mice defective in type I IFN receptors ($Ifnar1^{-/-}$ mice) are actually more resistant to systemic *Candida* infections [44]. This is also in line with our findings that PBMCs with the candidemia risk genotype in *IFIH1* tend to release more inflammatory cytokines. The hypothesis that MDA5 has a negative effect on the anti-*Candida* immune response has been proven by a very recent elegant study demonstrating that $Mda5^{-/-}$ mice are more resistant to disseminated candidiasis (Malireddi and Kanneganti, personal communication).

It is currently unclear which ligands cause activation of MDA5 in *Candida* infection. *Candida* is mainly recognized by cell surface pattern recognition receptors such as TLRs and C-type lectin receptors (CLRs), after which the fungus is internalized and subsequently digested in the phagolysosome [45, 46]. It is conceivable that, during this process, *Candida*-derived structures may leak from these organelles and enter the cytoplasm, a process described earlier for the recognition of mycobacterial peptidoglycans by the cytoplasmic receptor NOD2 [47–49]. Interestingly, a recent study has suggested that NOD2 is also important for the recognition of *Candida* chitin [50]. Nevertheless, there is currently no experimental evidence to support that either the wild-type form or a variant form of MDA5 has ligands other than the described RNAs.

In conclusion, this study demonstrates that the viral receptor MDA5 has an important role in modulating innate immune responses against the fungal pathogen *C. albicans*. Future research should shed light on the exact mechanisms through which MDA5 participates in the defense against the fungus. Nevertheless, the possible deleterious effects of MDA5-dependent stimulation during systemic candidiasis shown by our data suggest its potential usefulness as a novel therapeutic target.

**Acknowledgments** We thank Martin Oti for the helpful discussions. MJ and MGN were supported by an ERC Consolidator Grant (nr. 310372 to MGN). RvdL and MAH were supported by the Virgo Consortium, funded by the Dutch government (FES0908), and by the Netherlands Genomics Initiative (050-060-452). CW was supported by the ERC Advanced Grant, ERC-671274. XW was supported by NSFC 11101321 and NSFC 61263039 grants.

**Conflict of interest** The authors declare that they have no conflict of interest.

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