Early Stop Polymorphism in Human DECTIN-1 Is Associated with Increased Candida Colonization in Hematopoietic Stem Cell Transplant Recipients

Theo S. Plantinga,1,5a Walter J. F. M. van der Velden,2,6 Bart Ferwerda,1,5 Annemiek B. van Spriel,1a Gosse Adema,3 Ton Feuth,4 J. Peter Donnelly,2,5 Gordon D. Brown,4 Bart-Jan Kullberg,1,5 Nicole M. A. Blijlevens,2,5 and Mihai G. Netea1,5

Departments of 1Medicine, 2Haematology, 3Tumor Immunology, 4Epidemiology, Biostatistics and Health Technology Assessment, and 5Nijmegen Institute for Infection, Inflammation and Immunity (N4i), Radboud University Nijmegen Medical Centre, Nijmegen, the Netherlands; and 6Institute of Infectious Disease and Molecular Medicine, University of Cape Town, Cape Town, South Africa

(See the editorial commentary by Bochud and Calandra on pages 733–5)

Background. Intensive treatment of hematological malignancies with hematopoietic stem cell transplantation (HSCT) is accompanied by a high incidence of opportunistic fungal infection, but individual risk varies significantly. Dectin-1, a C-type lectin that recognizes 1,3-β-glucans from fungal pathogens, including Candida species, is involved in the initiation of the immune response against fungi.

Methods. Screening for the DECTIN-1 Y238X polymorphism within a group of 142 patients undergoing HSCT was correlated with Candida colonization and candidemia. Furthermore, functional studies were performed on the consequences of the polymorphism.

Results. Patients bearing the Y238X polymorphism in the DECTIN-1 gene were more likely to be colonized with Candida species, compared with patients bearing wild-type DECTIN-1, necessitating more frequent use of fluconazole in the prevention of systemic Candida infection. Functional assays demonstrated a loss-of-function phenotype of the polymorphism, as shown by the decreased cytokine production by immune cells bearing this polymorphism.

Conclusions. The Y238X polymorphism is associated with increased oral and gastrointestinal colonization with Candida species. This suggests a crucial role played by dectin-1 in the mucosal antifungal mechanisms in immunocompromised hosts. The finding that DECTIN-1 polymorphisms rendered HSCT recipients at increased risk for fungal complications may contribute to the selection of high-risk patients who should be considered for antifungal prophylaxis to prevent systemic candidiasis.

The treatment of patients with hematological malignancies with a hematopoietic stem cell transplantation (HSCT) following myeloablative conditioning is accompanied by complications that include mucosal barrier injury, prolonged neutropenia, and graft-versus-host disease (GvHD), all of which contribute to fungal and other opportunistic infections [1, 2]. Traditional risk factors are predictive for incidence of invasive fungal disease in general, but the individual risk is more difficult to determine, although this is needed for a more guided use of antifungal prophylaxis and therapy.

Dectin-1 is a member of the C-type lectin receptor family that recognizes the β-1,3-glucan motif of the cell wall of pathogenic fungi [3]. Dectin-1 is mainly expressed by immune cells of the myeloid lineage (neutrophils, macrophages, and dendritic cells). Several studies have shown that dectin-1 belongs to the armamentarium of immune cells against fungal pathogens, including Candida species and Aspergillus species [4, 5]. Furthermore, dectin-1 synergizes with TLR2 and TLR4 signals and promotes Th1 and Th17 responses to activate antifungal host defense [6–8].

Recently, we have demonstrated that a polymorphism in DECTIN-1 (Y238X, rs 16910526) is respon-
sible for recurrent mucocutaneous fungal infections in a Dutch family (unpublished data). This polymorphism resulted in an early stop codon, which leads to the loss of the last 10 amino acids of the extracellular domain, and in a diminished capacity to bind β-glucans.

To test the hypothesis that DECTIN-1 variants influence the susceptibility to fungal infection in general and to Candida infection in particular for HSCT recipients, we first assessed the frequency of DECTIN-1 polymorphisms in a healthy Dutch population. We identified the Y238X mutation as an important polymorphism and the only one with significant functional consequences for the recognition of Candida species. Subsequently, we have investigated the impact of this DECTIN-1 polymorphism on the incidence of mucosal Candida colonization and the occurrence of candidemia and other invasive fungal infections among 142 patients who received a sibling T cell–depleted allogeneic HSCT.

**PATIENTS, MATERIALS, AND METHODS**

*Genetic screening for DECTIN-1 polymorphisms.* DNA template of the DECTIN-1 gene (also named CLEC7A) was taken from GenBank, chromosome position 12p13, NC_000012.10. Sequencing of the exonic and nearby intronic regions of the DECTIN-1 gene in the 138 healthy volunteers was performed by applying the primers and conditions depicted in table 1. Genotyping for the presence of the Y238X polymorphism in the patient and donor groups was performed by applying the TaqMan single-nucleotide polymorphism (SNP) assay C_33748481_10 on the 7300 ABI Real-Time polymerase chain reaction system (Applied Biosystems). Patients, donors, and healthy volunteers had given informed consent to prospective collection of DNA samples for investigational use.

*Flow cytometry.* For staining of membrane-bound dectin-1, monocytes were incubated with 5 µg/mL murine anti–dectin-1 directed towards the stalk region (BD6) conjugated with biotin, or mouse immunoglobulin (Ig) G2b isotype control, followed by streptavidin–allophycocyanin conjugated goat anti-mouse antibody (Pharmingen). Dectin-1 expression was determined by flow cytometry (FACScalibur; BD Biosciences).

*Confocal microscopy.* Confocal laser scanning microscopy was performed as described by Meyer-Wentrup et al [9]. Cells were stained with 10 µg/mL mouse anti–dectin-1 (clone 259931; R&D Systems) or mouse IgG2b isotype control, followed by goat anti-mouse-Alexa647–conjugated secondary antibody (Molecular Probes). Samples were mounted in mowiol and analyzed by confocal laser scanning microscopy (Olympus FV1000).

*Cytokine stimulation assays.* Isolation of mononuclear cells was performed as described previously [6]. Cells were incubated at 37°C for the indicated duration (4 h or 48 h) with either culture medium or the various stimuli: 10^5 heat-killed Candida albicans (heat-killed by incubation at 56°C for 30 min), the TLR2 agonist Pam3Cys (10 µg/mL) and β-glucan (10 µg/mL) or a combination of Pam3Cys and β-glucan. In a part of the experiments, after peripheral blood mononuclear cell (PBMC) isolation, monocytes were purified by CD14+ MACS MicroBeads (Miltenyi Biotec) and stimulated as described above. Cytokine production was measured by enzyme-linked immunosorbent assay (R&D Systems).

**Patients and donors.** We performed a retrospective analysis involving 142 Dutch patients undergoing HSCT because of hematological malignancies. The patients and donors were consecutively admitted to our transplant unit from May 1996 through September 2007 for a human leukocyte antigen–identical sibling, partially T cell–depleted allogeneic HSCT. The characteristics of patients, donors, and HSCT procedures are given in table 2.

**Treatment protocol.** The treatment protocol has been described in detail elsewhere [10]. The conditioning regimen consisted of cyclophosphamide (60 mg/kg for 2 days) in combination with either total body irradiation (4.5 Gy for 2 days) or busulfan (4 mg/kg for 4 days). Idarubicin (42 mg/kg in 48 h) was often added to these conditioning regimen to reduce the risk of relapse in the setting of T cell–depleted HSCT [11]. On day 0, all patients were given an allogeneic HSCT containing 3.2 × 10⁶ CD34+ cells/kg (range, 0.6–11.6 CD34+ cells/kg) and 0.5 × 10⁶ CD3+ cells/kg (range, 0.1–0.8 CD3+ cells/kg).

Antimicrobial prophylaxis consisted of 500 mg of ciprofloxacin given twice daily and 500 mg of valaciclovir given 3 times daily. Surveillance cultures for Candida were collected twice weekly from hospital admission until hospital discharge, with the first culture samples obtained on the day of hospital admission, before the start of conditioning. Fluconazole (200 mg daily) was only prescribed to those who were colonized with C. albicans, Candida tropicalis, or Candida parapsilosis (not Candida krusei or Candida glabrata) when the yeast was present in both fecal cultures and mouth washes obtained on the same day, or when obtained from the same site on 2 consecutive occasions [12].

Invasive fungal infections were defined according to the European Organization for the Treatment of Cancer/Mycoses Study Group consensus guidelines, designating invasive fungal disease as possible, probable, or proven [13]. Oral mucositis was graded daily according to the validated Nijmegen Nursing Mucositis Scoring System (NNMSS) [14]. Acute GvHD was diagnosed by clinical signs or pathological examination of skin, gut, or liver biopsy samples and was graded according to the criteria of Glucksberg et al [15].

**Statistical analysis.** In multivariable logistic regression analyses, we investigated the association of the DECTIN-1 status of the patient with Candida colonization, controlling for underlying hematological disease, age, and sex of the patient. In
Table 1. Primers and Polymerase Chain Reaction Conditions Applied to Amplify Every Exon and Proximal Intronic Regions of the DECTIN-1 Gene to Perform Sequence Analysis

<table>
<thead>
<tr>
<th>Exon, primer</th>
<th>Sequence, 5'–3'</th>
<th>MgCl₂, mM</th>
<th>Annealing temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Forward: TTTACACGGTTAGCCAGCT</td>
<td>2.5</td>
<td>52°C</td>
</tr>
<tr>
<td></td>
<td>Reverse: GTGAAATAGTTTGCATGGTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Forward: CCGTTTATAAGTGAAATGGGC</td>
<td>1.75</td>
<td>60°C</td>
</tr>
<tr>
<td></td>
<td>Reverse: ACCGTGCAAGGCCAGATTTT</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Forward 1: GCCAGTGATAAATCAGTTACT</td>
<td>3.5</td>
<td>56°C</td>
</tr>
<tr>
<td></td>
<td>Reverse 1: TTCTTCTTCACCTTTCACT</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Forward 2: TGGCAACATTTTCCCTTCT</td>
<td>3.5</td>
<td>56°C</td>
</tr>
<tr>
<td></td>
<td>Reverse 2: GGCAAGGCGATAGTTAAAGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Forward: TCATTACCTGGAATCTCCCTCT</td>
<td>2.5</td>
<td>56°C</td>
</tr>
<tr>
<td></td>
<td>Reverse: TGGCAACTAATTGGTTATTTCA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Forward: GCTGCTCGACAGAGGTTTTC</td>
<td>1.75</td>
<td>62°C</td>
</tr>
<tr>
<td></td>
<td>Reverse: GGATGGTCTCGATCTCCTGA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Forward: AATCACAGCCTCTCCCTTCA</td>
<td>2.5</td>
<td>60°C</td>
</tr>
<tr>
<td></td>
<td>Reverse: GATTAAAGCCTCCTTTCCAA</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**NOTE.** For all amplicons, sequence analyses were carried out with the forward and the reverse primer. For the sequences of exon 3, technical difficulties were encountered. Therefore, we amplified exon 3 with 2 different primer pairs and aligned both forward assays after sequence analysis.

### RESULTS

**DECTIN-1 polymorphism screening in healthy individuals.** Genetic variation in the DECTIN-1 gene was investigated for 138 Dutch healthy volunteers and revealed the polymorphisms shown in figure 1. A small number of polymorphisms were identified, with the Y238X (rs16910526) in exon 6 being the only exonic polymorphism. This polymorphism was present in 19 (13.8%) of 138 individuals, all of whom were heterozygous, resulting in an allele frequency of 6.9%. This polymorphism was identified earlier in a family that had previously been analyzed for mucocutaneous Candida infection (unpublished data); 3 members of this family were homozygous for the Y238X polymorphism.

**Dectin-1 protein expression.** We investigated the consequence of the polymorphism on dectin-1 expression and localization at the protein level. Monocytes isolated from individuals who were homozygous for the wild-type DECTIN-1 allele and from individuals heterozygous or homozygous for the Y238X polymorphism were analyzed for dectin-1 expression by flow cytometry and confocal microscopy. Monocytes from the individuals homozygous for the Y238X polymorphism...
Table 2. Clinical Characteristics of the Study Group

<table>
<thead>
<tr>
<th>Variable</th>
<th>Homozygous wild-type for DECTIN-1</th>
<th>Heterozygous for DECTIN-1 Y238X</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of recipients</td>
<td>126</td>
<td>15</td>
<td></td>
</tr>
<tr>
<td>No. of donors</td>
<td>116</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Male sex, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recipients</td>
<td>65</td>
<td>60</td>
<td>.78</td>
</tr>
<tr>
<td>Donors</td>
<td>56</td>
<td>64</td>
<td>.64</td>
</tr>
<tr>
<td>Age, mean years (range)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recipients</td>
<td>47.5 (18.5–64.4)</td>
<td>42.8 (19.2–59.8)</td>
<td>.11</td>
</tr>
<tr>
<td>Donors</td>
<td>47.4 (14–75.6)</td>
<td>43.3 (23.8–67.6)</td>
<td>.24</td>
</tr>
<tr>
<td>Diagnosis</td>
<td></td>
<td></td>
<td>.79</td>
</tr>
<tr>
<td>AML/ALL</td>
<td>60 (47.6)</td>
<td>8 (53.3)</td>
<td></td>
</tr>
<tr>
<td>CML/MPS</td>
<td>26 (20.6)</td>
<td>3 (20)</td>
<td></td>
</tr>
<tr>
<td>MDS</td>
<td>23 (18.3)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymphoma/CLL</td>
<td>17 (13.5)</td>
<td>4 (26.7)</td>
<td></td>
</tr>
<tr>
<td>Conditioning regimen</td>
<td></td>
<td></td>
<td>.25</td>
</tr>
<tr>
<td>Ida-Cyclo-TBI</td>
<td>79 (62.7)</td>
<td>10 (66.7)</td>
<td></td>
</tr>
<tr>
<td>Ida-Cyclo-Bus</td>
<td>15 (11.9)</td>
<td>1 (6.7)</td>
<td></td>
</tr>
<tr>
<td>Cyclo-TBI</td>
<td>27 (21.4)</td>
<td>1 (6.7)</td>
<td></td>
</tr>
<tr>
<td>Cyclo-Bus</td>
<td>5 (4.0)</td>
<td>3 (20)</td>
<td></td>
</tr>
<tr>
<td>TBI</td>
<td>106 (84.1)</td>
<td>11 (73.3)</td>
<td></td>
</tr>
<tr>
<td>Stem cell source</td>
<td></td>
<td></td>
<td>&gt;.99</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>72 (57.1)</td>
<td>9 (60.0)</td>
<td></td>
</tr>
<tr>
<td>Bone marrow</td>
<td>54 (42.9)</td>
<td>6 (40.0)</td>
<td></td>
</tr>
<tr>
<td>T cell depletion</td>
<td></td>
<td></td>
<td>.43</td>
</tr>
<tr>
<td>CD34 selection</td>
<td>61 (48.5)</td>
<td>9 (60.0)</td>
<td></td>
</tr>
<tr>
<td>Counterflow elutriation</td>
<td>41 (32.5)</td>
<td>4 (26.7)</td>
<td></td>
</tr>
<tr>
<td>CD3/CD19 selection</td>
<td>24 (19.0)</td>
<td>2 (13.3)</td>
<td></td>
</tr>
<tr>
<td>Duration of neutropenia,a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mean days (range)</td>
<td>11.9 (6–20)</td>
<td>11.0 (6–15)</td>
<td>.23</td>
</tr>
<tr>
<td>Acute GvHD</td>
<td></td>
<td></td>
<td>.77</td>
</tr>
<tr>
<td>Grade 0–I</td>
<td>81 (64.3)</td>
<td>11 (73.3)</td>
<td></td>
</tr>
<tr>
<td>Grade II-IV</td>
<td>39 (31.0)</td>
<td>4 (26.7)</td>
<td></td>
</tr>
<tr>
<td>Grade III-IV</td>
<td>10 (7.9)</td>
<td>1 (6.7)</td>
<td></td>
</tr>
<tr>
<td>NA</td>
<td>6 (4.7)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NOTE. Data are no. (%) of patients, unless otherwise indicated. All patients received a human leukocyte antigen–matched sibling partially T cell–depleted hematopoietic stem cell transplant. Graft-versus-host disease (GvHD) prophylaxis consisted of only cyclosporine for all patients. Differences between the study groups were compared using the Pearson χ² test or Fisher’s exact test and with the use of the independent Student’s t test where appropriate. No statistically significant differences were obtained. AML/ALL, acute myeloid and lymphatic leukemia; Bus, busulphan; CLL, chronic lymphatic leukemia; CML/MPS, chronic myeloid leukemia/myeloproliferative syndrome; Cyclo, cyclophosphamide; Ida, idarubicin; MDS, myelodysplastic syndrome; NA, not applicable; TBI, total body irradiation.

a Neutropenia was defined as ≤ 0.1 × 10⁹ cells/L.

exhibited no dectin-1 expression on the cell surface, whereas cells from individuals heterozygous for the Y238X polymorphism had intermediate cell surface expression, compared with cells from individuals with the wild-type allele (figure 2A). In line with this, no cell surface expression of dectin-1 could be detected on monocytes isolated from individuals who were homozygous for the Y238X polymorphism, in contrast with wild-type cells, when monocytes were analyzed by confocal microcopy (figure 2B). However, dectin-1 mRNA expression was demonstrated to be equal between the genotypes (data not shown). Thus, these findings demonstrate that dectin-1 protein expression is absent from the cell membrane of monocytes from individuals who are homozygous and intermediate on monocytes from individuals who are heterozygous for the Y238X polymorphism.

**Cytokine production.** Functional consequences of the
Y238X polymorphism were investigated in monocytes and
PBMCs isolated from individuals bearing only the wild-type
DECTIN-1 allele and individuals who were heterozygous or
homozygous for the Y238X polymorphism. Interleukin (IL–
1β induction by C. albicans was lower in cells from individuals
bearing the Y238X polymorphism (figure 3A; \( P < .05 \)). Dectin-
1 has been previously demonstrated to amplify TLR2 signaling
[6]; this effect was absent in cells isolated from individuals
homozygous for the Y238X allele (figure 3B). In contrast, IL-
18 and interferon (IFN–γ production was not defective in cells
isolated from these individuals (figure 3C; \( P = \) not significant).
Although a tendency toward a lower IL-18 production capacity
has been observed in cells from individuals heterozygous for
Y238X, compared with cells bearing only the wild-type DECTIN-
1 allele, this did not reach statistical significance. IL-18
production in cells from individuals homozygous for the Y238X
mutation was similar to production in cells from individuals
with the wild-type allele. Furthermore, IFN–γ production ca-
pacity is practically equal between the genotypes.

**DECTIN-1 Y238X polymorphism in patients and donors.**
The DECTIN-1 genetic status could be determined for 141
patients and 138 donors. Insufficient amounts of DNA pre-
cluded the determination of genetic status in 1 patient and 4
donors. Fifteen (10.6%) of 141 patients and 22 (15.9%) of 138
donors had the DECTIN-1 Y238X polymorphism; all individ-
uals were heterozygous. In 9 patient-donor pairs, both indi-
viduals were heterozygous for the polymorphism. There were
no statistically significant differences between the clinical char-
acteristics of patients with and patients without the DECTIN-
1 Y238X polymorphism (table 2). No difference was detected
in the severity of mucositis between patients with and patients
without the DECTIN-1 Y238X polymorphism, with a mean
NNMSS score of 3.8 vs 3.9 on day 0 and 7.2 vs 7.6 on day 7
(\( P = \) not significant).•

**Candida species colonization.** Seven patients who received
secondary antifungal prophylaxis at hospital admission were
excluded from the study, as were 11 patients with missing col-
onization data, leaving 124 patients eligible for analysis of Can-
dida colonization. At hospital admission, 46 (37.1%) of 124
patients were colonized with Candida species. Patients who
were heterozygous for the Y238X polymorphism were statisti-
cally significantly more often colonized than were patients
with DECTIN-1 wild-type alleles (11 [84.6%] of 13 vs 35
[31.5%] of 111; \( P < .001 \)) (odds ratio [OR], 11.9; 95% confi-
dence interval [CI], 2.5–56.8). After adjusting for diagnosis, the
OR was 12.2 (95% CI, 2.5–59.7); after adjusting for patient age
and sex, the OR was 12.0 (95% CI, 2.5–57.1). On the day of
HSCT (day 0), this difference persisted: 12 (92.3%) of 13 pa-
tients vs 50 (45.1%) of 111 patients (\( P = .001 \)). The unadjusted
OR was 14.6 (95% CI, 1.8–116.5) and the OR adjusted for
diagnosis, patient age, and sex was 15.5 (95% CI, 1.9–125.6).

Patients with a DECTIN-1 polymorphism were more likely
than other patients to receive fluconazole (9 [69.2%] of 13 vs
42 [37.8%] of 111; \( P = .03 \) ) (table 3). Among those patients
who received fluconazole, eradication was achieved in 1
(11.1%) of 9 patients who were heterozygous for the Y238X
polymorphism, compared with 14 (33.3%) of 42 patients who
bore only the wild-type DECTIN-1 allele (\( P = .25 \)). Colonizing
species were C. albicans (87% of patients), C. glabrata (8.1%),
and sporadically, C. krusei, Candida kefyr, Candida parapsilosis,
and Candida dublinensis (1.6% each). No difference in the fre-
frequency of colonization with particular Candida species was
observed between patients who bore heterozygous for Y238X
and patients who bore only the wild-type DECTIN-1 allele.

**Invasive fungal disease.** Patients who received early anti-
fungal prophylaxis with either itraconazole, voriconazole, or
posaconazole, starting from day 0 or earlier and until day 21,
were excluded (18 patients, 7 of whom had received secondary
prophylaxis and 11 of whom had participated in antimicrobial
studies). The overall incidence of candidemia until day 21 was

![Figure 1. Schematic drawing of the DECTIN-1 gene (also known as CLEC7A), consisting of an N-terminal cytoplasmatic tail (Cyto), encoded by exon 1, a transmembrane region (TM; exon 2), a stalk region (exon 3), and the carbohydrate recognition domain (CRD; exon 4–6). Intronic polymorphisms were detected in 5'UTR, intron 2, intron 3, exon 6, and 3'UTR. All variations are depicted together with their corresponding chromosome location and rs number, if available.](http://cid.oxfordjournals.org/Downloadedfrom)
Figure 2. Flow cytometry graphs of extracellular dectin-1 staining (A) and (B) fluorescent confocal staining of dectin-1 (right panel) and light microscopic image (left panel) on unstimulated monocytes derived from individuals homozygous for the wild-type DECTIN-1 allele (WT) and heterozygous (HET) or homozygous (HOM) for the DECTIN-1 Y238X polymorphism.

8.9% (11 of 123 patients). There was no statistically significant difference in the incidence of early candidemia between patients with and patients without the DECTIN-1 Y238X polymorphism (2 [18.2%] of 11 vs 9 [8.0%] of 112; \( P = .26 \)) (table 3); the OR was 2.5 (95% CI, 0.5–13.6). However, this study was not designed to detect differences in the risk of developing invasive candidiasis, because patients who were colonized were prescribed fluconazole to prevent systemic Candida infection, and the study was underpowered to find a difference.

Candidemia was caused by *C. albicans* in 7 of 11 cases, with the remainder of the cases being due to *C. glabrata* (1 case), *C. parapsilosis* (2), and *C. dublinesis* (1). Eight (15.7%) of 51 patients who were colonized and received fluconazole experienced candidemia. Two (3.6%) of 55 patients who were not colonized and who therefore received no fluconazole developed candidemia.

The incidence of proven and probable invasive mold infection up to day 100 was 3.3% (4 of 120 patients), with 4 probable and no proven mold infections. Three probable cases were due to *Aspergillus* species, and 1 probable case was due to *Rhizomucor* species. There was no statistically significant difference between pairs with and without a DECTIN-1 Y238X polymorphism; 1 (5.0%) of 20 patients with the Y238X polymorphism had a probable case, compared with 3 (3.0%) of 100 patients with the DECTIN-1 wild-type allele (\( P = .52 \)) (table 3). However, the study was underpowered to detect a difference, especially because of the very low incidence of mold infection.

DISCUSSION

In this study, we demonstrate that a newly characterized polymorphism in DECTIN-1 is associated with increased suscep-
Figure 3. Cytokine production capacity of interleukin (IL)-1β, IL-18, and interferon (IFN)-γ after stimulation of monocytes during 4 h (A) or peripheral blood mononuclear cells (PBMCs) during 48 h with β-glucan, Pam3Cys, or β-glucan/Pam3Cys (B) or with heat-killed Candida albicans (C). Cells were obtained from individuals with the wild-type allele (WT; n = 5) or individuals heterozygous (HET; n = 4) or homozygous (HOM; n = 3) for the DECTIN-1 Y238X polymorphism. Data are percentages compared with WT (± standard deviation). *P < .05.

Susceptibility to fungal colonization among HSCT recipients. As a consequence, among patients who were immunocompromised as a result of HSCT, the need to prescribe fluconazole to prevent systemic Candida infection was, in part, defined by the presence of this polymorphism.

Dectin-1 is one of the most important pattern recognition receptors for fungal pathogens in general and Candida species, in particular. Polymorphisms in pattern recognition receptors are known to be associated with an increased susceptibility to fungal infection [17, 18]. We hypothesized that genetic variants of DECTIN-1 could influence susceptibility to Candida colonization and infection in HSCT recipients.

Screening of all 6 exons of the DECTIN-1 gene in a healthy Dutch population revealed 1 exonic polymorphism and several intronic SNPs. Because the intronic SNPs are not likely to affect dectin-1 function, we considered the Y238X polymorphism to be the only polymorphism that could alter dectin-1 function and could influence susceptibility to fungal infection.
To characterize the functional consequences of the Y238X polymorphism in more detail, flow cytometry and confocal microscopy were performed. Expression of dectin-1 was absent on the cell membrane of cells isolated from individuals who were homozygous for the Y238X allele, which suggested a defective transport of the mutated form of dectin-1 to the cell membrane. Accordingly, dectin-1 expression was intermediate on cells from individuals who were heterozygous for the polymorphism.

After stimulation with heat-killed *C. albicans* or β-glucan, IL-1β secretion was intermediate in cells isolated from individuals who were heterozygous for the Y238X polymorphism and low in cells from individuals who were homozygous for this polymorphism, compared with secretion in cells from individuals who were homozygous for the wild-type allele. Moreover, the previously described synergism between TLR2 and dectin-1 signals [6] was completely absent in individuals homozygous for the Y238X polymorphism. These data demonstrate the loss-of-function effect of the Y238X polymorphism for dectin-1 activity. However, no difference in the production of IL-18 and IFN-γ could be observed between the different DECTIN-1 genotypes. This could well be because of a certain redundancy of dectin-1 signaling in production of these cytokines, and this is accompanied by a normal *Candida*-killing activity by neutrophils from individuals bearing the Y238X allele, which suggested a defective transport of the mutated form of dectin-1 to the cell membrane. Accordingly, dectin-1 expression was intermediate on cells isolated from individuals who were heterozygous for the polymorphism.

In the follow-up of these genetic and immunological studies, we demonstrate that this polymorphism has a significant impact on oral and gastrointestinal mucosal colonization with *Candida* species in HSCT recipients, which defined the need for the use of fluconazole in our approach. This is in line with the finding of recurrent mucocutaneous candidiasis in a family with individuals who were homozygous for the DECTIN-1 polymorphism (unpublished data). The overall rate of fungal colonization at hospital admission was comparable to that in earlier studies, with colonization rates of 28%–57% [19, 20]. These data strongly support a role of dectin-1 mediated mechanisms for mucosal anti-*Candida* defense.

The mechanisms of the increased susceptibility to mucosal colonization with *Candida* in individuals bearing the DECTIN-1 polymorphism cannot be definitively pinpointed, although the defective cytokine responses are probably involved. Although monocytes are important for mucosal defenses, epithelial cells may also contribute to these effects. Interestingly, the expression of dectin-1 on epithelial cells has been recently demonstrated, and the interaction of these cells with fungal pathogens leads to chemokine release [21]. One has to consider, however, that in the setting of HSCT, both monocytes and epithelial cells of the mucosa are damaged for a prolonged period, although they are largely intact at hospital admission and during the first days of conditioning. On the other hand, the resident macrophages of the mucosa are less affected, and it is most likely that the impact of the DECTIN-1 polymorphism is exerted at their level.

The presence of the DECTIN-1 polymorphism did not result in a significantly higher incidence of candidemia, although that might have been expected, because mucosal colonization is associated with systemic candidiasis in HSCT recipients who experience mucosal barrier injury [22, 23]. Importantly, in this cohort, patients were prescribed oral fluconazole when colonized, which is known to reduce the incidence of candidemia [20, 24]. Therefore, fluconazole was most likely a confounding factor, precluding a definitive conclusion regarding the role of the DECTIN-1 Y238X polymorphism for susceptibility to early candidemia, although our data may suggest an increased risk (OR, 2.5; 95% CI, 0.5–13.6). In addition, our study was underpowered to study the impact of the polymorphism on the occurrence of invasive mold infections because of the low incidence of these infections.

In our culture-guided approach, fluconazole could safely be withheld for those individuals who were not colonized with
Candida species, preventing overtreatment and accompanied adverse effects and costs. However, those individuals who were colonized remained at significant risk for candidemia, necessitating better ways to predict, determine, and treat colonization at an early stage. Determining the DECTIN-1 status before HSCT might be a factor contributing to a more risk-adapted prophylactic approach. Because Candida colonization is associated with invasive disease among HSCT recipients, we propose that patients bearing the polymorphism should be considered for antifungal prophylaxis to prevent systemic candidiasis. Nevertheless, future studies in prospective trials are necessary to further confirm the impact of this newly characterized DECTIN-1 polymorphism and to define its role in selecting adequate prophylaxis or early treatment for HSCT recipients.

Acknowledgments

Financial support. M.G.N. was supported by a Vidi grant of the Netherlands Organization for Scientific Research.

Potential conflicts of interest. All authors: no conflicts.

References

18. van der Graaf CA, Netea MG, Morre SA, et al. Toll-like receptor 4 Asp299Gly/Thr391Ile polymorphisms are a risk factor for Candida bloodstream infection. Eur Cytokine Netw 2006; 17:29–34.