

Contribution of Genetic and Clinical Risk Factors to Development of Candidemia in Patients Receiving Home Parenteral Nutrition

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Abstract

Background: Patients receiving home parenteral nutrition (HPN) have an increased risk for central line–associated bloodstream infections (CLABSIs), including candidemia. Recently, 7 single-nucleotide polymorphisms (SNPs) in *TLR1*, *CD58*, *LCE4A-Clorf68*, and *TAGAP* have been associated with the development of candidemia. Identification of host-genetic as well as clinical risk factors may help to identify patients who have an increased susceptibility to such infections. The aim of this study was to investigate the relevance of the reported SNPs in patients receiving HPN, and to explore clinical risk factors associated with candidemia. **Methods:** We analyzed blood samples of adult patients who started HPN between 1976 and 2017 at our referral center for intestinal failure. Primary outcome was the association between *TLR1*, *CD58*, *LCE4A-Clorf68*, or *TAGAP* SNPs and candidemia. Secondary outcomes included the relation between severity of infection and these SNPs, and clinical risk factors for candidemia. **Results:** Of 341 included patients, 42 (12%) experienced a candidemia (range 1–6). None of the 7 SNPs were associated with candidemia or the severity of infection. The rate of non-*Candida*-related CLABSIs was significantly associated with candidemia (rate ratio, 1.29; 95% CI, 1.14–1.46; $P < 0.001$). **Conclusions:** None of 7 known SNPs in *TLR1*, *CD58*, *LCE4A-Clorf68*, or *TAGAP* were associated with candidemia or severity of infection in patients receiving HPN. The rate of non-*Candida*-related CLABSIs was significantly associated with the development of candidemia. The latter supports the key role of aseptic catheter handling with respect to *Candida* susceptibility in patients receiving HPN. (*JPEN J Parenter Enteral Nutr.* 2020;44:282–290)

Keywords

candidemia; *CD58*; central line–associated bloodstream infection; fungemia; home parenteral nutrition; intestinal failure; *LCE4A-Clorf68*; susceptibility; *TAGAP*; *TLR1*

Clinical Relevancy Statement

In patients receiving home parenteral nutrition (HPN), approximately 8%–15% of central line–associated bloodstream infections (CLABSIs) are caused by *Candida* species.

Several clinical risk factors for candidemia have been identified in previous studies. In addition, 7 single-nucleotide polymorphisms (SNPs) in *TLR1*, *CD58*, *LCE4A-Clorf68*, and *TAGAP* have been recently associated with an increased risk for candidemia. Identification of both host-genetic

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and clinical risk factors may help to identify patients receiving HPN who have an increased susceptibility to candidemia and guide patient-specific preventive strategies. To date, however, studies exploring genetic risk factors in patients receiving HPN are extremely scarce, and no studies have been performed investigating genetic risk factors for candidemia.

This retrospective cohort study was conducted to determine the association between *TLR1*, *CD58*, *LCE4A-Clorf68*, or *TAGAP* SNPs and candidemia in patients receiving HPN, and to explore clinical risk factors for candidemia. Although none of 7 SNPs were associated with candidemia, the rate of non-*Candida*-related CLABSIs was significantly associated with the development of candidemia. This emphasizes the importance of catheter care to prevent candidemia in clinical practice.

Introduction

Patients with intestinal failure (IF) depend on long-term intravenous supplementation of nutrition and/or fluids in the home setting. This treatment strategy is coined as home parenteral nutrition (HPN) support, and it centers on self-management of central venous access devices (CVADs) by patients or their caregivers. Patients receiving HPN have an increased risk for central line-associated bloodstream infections (CLABSIs), 8%–15% of which are caused by *Candida* species.^{1–3} Candidemia is infamously known for its substantial morbidity and mortality rates of up to 40%, also due to the tendency for metastatic spread, with an obvious impact on patient quality of life and healthcare resources.^{4–11} To prevent dissemination of fungal pathogens, timely removal of CVADs is crucial.^{9,12} Repeated removal of CVADs, however, eventually compromises the remaining options to obtain reliable venous access. Therefore, measures to prevent CLABSIs, including candidemia, are of key importance to maintain venous access.

In addition to the presence of CVADs, other clinical risk factors for invasive candidiasis include recent surgery (especially in the case of anastomotic leakage), critical illness, transplant procedures, and the use of immunosuppressive drugs and broad-spectrum antibiotics.⁹ These risk factors, however, do not explain all of the variation in susceptibility to *Candida* infections. Hence, it is assumed that host-genetic risk factors contribute to the development of candidemia as well. Indeed, Plantinga et al recently identified 3 single-nucleotide polymorphisms (SNPs) (rs5743611, rs4833095, and rs5743618) in the Toll-like receptor 1 (*TLR1*) gene in a relatively large cohort of 245 patients who developed candidemia.⁶ *TLR1* plays a central role in the host defense against infections. Microbial products activate *TLR1*, which results in a burst of proinflammatory cytokines with simultaneous activation of the innate immune system.¹³ The SNPs in *TLR1* seem to decrease this proinflammatory

cytokine release, in particular of interleukin (IL)-1 β , IL-6, and IL-8.⁶

In another study of 217 candidemia patients, Kumar et al analyzed 118,989 SNPs in 186 loci related to immune-mediated diseases.¹⁴ The authors identified 4 SNPs (rs17035850, rs12025416, rs4845320, and rs3127214) that conferred an increased risk for candidemia. The SNPs rs17035850 and rs12025416 are both located near the *CD58* gene, an important factor in mediating *Candida* phagocytosis, inhibiting germination, and modulating *Candida*-specific cytokine production.¹⁴ The third SNP, rs4845320, is located at the *LCE4A-Clorf68* locus and is involved in epithelial barrier function.^{14,15} Finally, rs3127214 is located at the 5' end of *TAGAP* and encodes for T-cell activation RhoGTPase-activating protein.¹⁶ *TAGAP* has a role in *Candida*-induced inflammation and antifungal host defense.¹⁴ Interestingly, patients with 2 or more risk alleles had a 19.4-fold increase in risk for developing candidemia.

The identification of both host-genetic and clinical risk factors may help to identify patients with HPN who have an increased susceptibility to candidemia and guide patient-specific preventive strategies. For example, these patients might benefit from prophylactic antifungal therapy or adjuvant immunotherapy. In addition, arteriovenous fistulas, which have been associated with extremely low CLABSI rates, might be created preemptively as an alternative to CVADs for parenteral nutrition administration.^{9,17}

It remains unclear, however, whether the previously mentioned SNPs play a similar role in patients with HPN and which clinical factors are involved in the development of candidemia. This notion urged us to assess the impact of the reported SNPs in our cohort of patients with HPN, and to explore clinical risk factors associated with candidemia.

Subjects and Methods

Ethics Statement

This study has been approved by the ethics committee of the Radboud University Medical Center (reference number 2018–4597). Formal informed consent was waived by the ethical committee. This study has been conducted in accordance with the Declaration of Helsinki.

Study Design and Population

We performed a case vs disease-matched control study, in which we analyzed available historically collected whole blood samples from adult patients receiving HPN at our referral center for IF (Figure S1). Identified patients were cross-checked for clinical data from the Nijmegen IF Registry, a retrospective, single-center, Web-based database comprising IF patients who have been under treatment for IF since 1976.¹⁸ To be included, patients had to have a whole

blood sample available and had to have received at least 2 months of parenteral nutrition and/or fluids. The following data were used: patient characteristics (sex, age at start of HPN, pathophysiological mechanism of IF, underlying disease, diabetes status, presence of a stoma, transplant status, use of immunosuppressive therapy), time receiving HPN, and the number of candidemia and non-*Candida*-related CLABSIs.

Outcomes and Definitions

Primary outcome was the association between SNPs in *TLR1*, *CD58*, *LCE4A-Clorf68*, or *TAGAP* and candidemia. Predefined secondary outcomes included the association of SNPs in *TLR1*, *CD58*, *LCE4A-Clorf68*, or *TAGAP* and candidemia rates (number of candidemia per 1000 catheter days), and severity of infection (persistent candidemia or disseminated *Candida* infection). Finally, we explored clinical risk factors associated with candidemia.

Both candidemia and non-*Candida*-related CLABSIs were based on the current Centers for Disease Control and Prevention guidelines for surveillance of bloodstream infections.^{19,20} A CLABSI required the following set of conditions: (1) presence of a systemic infection or sepsis (eg, fever, hypotension, and/or chills), (2) a recognized pathogen species cultured from ≥ 1 blood culture, (3) the cultured microorganism was not related to an infection site other than the CVAD, and (4) in case of a common commensal (eg, coagulase-negative *Staphylococcus* species), > 1 blood culture had to be positive from the CVAD and/or peripheral vein.

Patients were classified as having a persistent candidemia when they had positive blood cultures for *Candida* species ≥ 5 days during follow-up, despite adequate antifungal therapy. A disseminated *Candida* infection was defined as the presence of *Candida* species at normally sterile body sites other than the bloodstream or urine during follow-up. Use of immunosuppressive medication was defined as $> 50\%$ of follow-up period on systemic non-chemotherapeutic drugs that suppress or reduce immune function.

DNA Isolation

DNA was isolated from venous whole blood in 10 mL Monoject tubes containing 18.0 mg EDTA (BD, Plymouth, United Kingdom) using the High Pure PCR Template Preparation Kit (Roche Diagnostics GmbH, Mannheim, Germany) according to the manufacturer's protocol. DNA samples were subsequently stored at 4°C–8°C until further use.

Genotyping

Specific sets of primers and probes were designed by using Beacon Designer 7.0 software (PREMIER Biosoft, Palo

Alto, CA, USA). The sequences of the primers and probes were checked for polymorphisms in their binding sites using SNPCheck version 3 and are shown in Table S1.²¹ The 6-fluorescein amidite and hexachloro-fluorescein amidite were covalently bound to the 5'-end of the probes. The Black Hole Quencher-1 was bound to the 3'-end of the probes. Primers and probes were prepared by Sigma-Aldrich Chemie BV (Zwijndrecht, the Netherlands). All SNPs were genotyped by means of real-time polymerase chain reaction (PCR) techniques with the CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA). Quality control was performed by duplicating samples within and across plates and by the incorporation of positive and negative control samples. Results were analyzed with data analysis software Bio-Rad CFX Manager 3.0 (Bio-Rad Laboratories).

Statistical Analysis

Continuous variables were presented as medians and interquartile ranges (IQR). Deviation from Hardy-Weinberg equilibrium for all 7 SNPs were calculated with the use of a χ^2 test for patients with and without candidemia, separately. Pairwise linkage disequilibrium, D' , and r^2 were calculated using Haploview. Haplotype blocks were assigned using a D' confidence interval algorithm from Gabriel et al.²² Logistic regression analyses were used to calculate odds ratios (ORs) with 95% CIs for genotypes associated between patients with and without candidemia, and with or without persistent candidemia or disseminated *Candida* infections, using a dominant model (ie, minor allele homozygote combined with the heterozygote as risk genotypes). In a secondary analysis, corrections were performed for multiple candidemias per patient and time receiving HPN, by using a random effect Poisson regression model. These results were presented as candidemia rates (number of candidemia per 1000 catheter days) between genotypes, and differences were compared with rate ratios and 95% CIs. For both the logistic regression and Poisson regression analyses, correction for significant different baseline characteristics or a change of $\geq 10\%$ on unadjusted estimates by covariates took place. Random effect Poisson regressions analysis was also used to identify clinical risk factors for candidemia. Potential risk factors included sex, age at start of HPN, pathological mechanism of IF, diabetes mellitus, presence of a stoma, use of immunosuppressive drugs, transplant status, and a patient's non-*Candida*-related CLABSI rate. Risk factors that showed in the univariable Poisson regression analysis a P -value ≤ 0.2 were included in the final multivariable Poisson regression analysis. Results with a P -value < 0.05 were considered statistically significant. Patients were excluded from relevant (sub)analyses in case of missing data. All statistical analyses were performed with SPSS statistical software package version 22.0 (SPSS, Chicago, IL, USA) or

Table 1. Baseline Characteristics of Patients With and Without a Candidemia.

Characteristics	No Candidemia (n = 299)	Candidemia (n = 42)	P-Value
Female, no. of patients (%)	206 (69)	30 (71)	0.74
Age at start HPN, median years (IQR)	52 (42–63)	44 (34–57)	0.14
Medical condition, no. of patients (%)			0.72
Short bowel syndrome	148 (50)	16 (38)	
Gastrointestinal motility disorder	102 (34)	18 (43)	
Mechanical obstruction	12 (4)	2 (5)	
Small bowel mucosal disease	14 (5)	3 (7)	
Intestinal fistula	13 (4)	1 (2)	
Other	10 (3)	2 (5)	
Underlying disease, no. of patients (%)			0.84
Intestinal dysmotility (primary/idiopathic)	59 (20)	12 (29)	
Intestinal dysmotility (secondary)	43 (14)	6 (14)	
Crohn's disease	48 (16)	5 (12)	
Mesenteric ischemia	44 (15)	4 (10)	
Surgical complications	24 (8)	2 (5)	
Extrinsic mechanical obstruction	10 (3)	1 (2)	
Radiation enteritis	7 (2)	1 (2)	
Other	64 (21)	11 (26)	
Presence of a stoma	160 (54)	24 (57)	0.66
Transplantation	11 (4)	0 (0)	0.21
Kidney	4	0	
Stem cell	4	0	
Intestines	2	0	
Kidney and intestines	1	0	
Diabetes, no. of patients (%)	20 (7)	3 (7)	>0.99
Non- <i>Candida</i> -related CLABSI history, no. of patients (%) ^a	149 (50)	34 (81)	
Non- <i>Candida</i> -related CLABSI rate, median (IQR) ^a	0 (0–1.31)	1.32 (0.54–2.91)	<0.001
Drug use, no. of patients (%)			
Immunosuppressives ^b	54 (18)	8 (19)	0.73
Unknown	3 (1)	1 (2)	

(continued)

Table 1. (continued)

Characteristics	No Candidemia (n = 299)	Candidemia (n = 42)	P-Value
<i>Candida</i> species cultured (%) ^c		65	
<i>Candida albicans</i>		36 (55)	
<i>Candida parapsilosis</i>		11 (17)	
<i>Candida glabrata</i>		9 (14)	
<i>Candida tropicalis</i>		4 (6)	
<i>Candida krusei</i>		2 (3)	
<i>Candida dubliniensis</i>		2 (3)	
Unknown <i>Candida</i> species		1 (2)	

CLABSI, central line-associated bloodstream infection; HPN, home parenteral nutrition; IQR, interquartile range.

^aAll microorganisms other than *Candida* species causing a CLABSI were included. Non-*Candida*-related CLABSI rate is expressed as number of CLABSIs per 1000 catheter days.

^bImmunosuppressive medication comprises systemic non-chemotherapeutic drugs that suppress or reduce immune function. For example, prednisolone, methotrexate, or adalimumab.

^cOne patient experienced a candidemia episode with both a *C. glabrata* and *C. Krusei*. A second patient experienced a candidemia episode with both a *C. parapsilosis* and *C. dubliniensis*.

R software version 3.2.4 (The R Foundation for Statistical Computing) for the Poisson regression analyses.

Results

In total, 341 (76%) of 451 patients receiving HPN were included in the analyses (Figure S1). Of these, 42 (12%) patients experienced 1 or more candidemia episodes (range 1–6). Baseline characteristics of patients are shown in Table 1. The median follow-up time of patients without candidemia was 3.5 years (IQR 1.6–7.1) and for candidemia patients 5.3 years (IQR 2.3–8.0). In total, 63 candidemia episodes and 572 non-*Candida*-related CLABSIs occurred during 673,278 catheter days. The overall candidemia rate was 0.09 infections per 1000 catheter days (95% CI 0.07–0.12). The median time to first candidemia was 227 days (IQR 43–927). Eighteen (29%) candidemia developed within 30 days following a CLABSI. In total, 28 (44%) candidemia developed while the patient received broad-spectrum antibiotics. Of the 42 patients with a candidemia, 17 (40%) had a surgical intervention within 30 days before the initial candidemia diagnosis. Of these, 16 had a venous access device replacement and 1 underwent major abdominal surgery. Five (12%) patients had been admitted to the intensive care unit within 30 days prior to candidemia diagnosis. Eventually, 2 (5%) patients died within 30 days after the candidemia diagnosis. Cultured *Candida* species are shown in Table 1.

Table 2. Genetic Analysis of Patients With and Without Candidemia.

Gene/ Locus	SNP	Genotype	No Candidemia (%) (n = 299)	Candidemia (%) (n = 42)	Adjusted Odds Ratio (95% CI) ^a	P-Value
<i>TLR1</i>	rs5743611	Homozygous (G/G)	252 (84)	34 (81)	Reference	0.66
		Heterozygous (G/C)	44 (15)	7 (17)	1.21 (0.52–2.85)	
		Homozygous (C/C)	3 (1)	1 (2)		
	rs4833095	Homozygous (A/A)	158 (53)	24 (57)	Reference	0.69
		Heterozygous (A/G)	119 (40)	17 (41)	0.88 (0.45–1.71)	
		Homozygous (G/G)	22 (7)	1 (2)		
	rs5743618	Homozygous (G/G)	146 (49)	18 (43)	Reference	0.34
		Heterozygous (G/T)	121 (41)	23 (55)	1.39 (0.71–2.73)	
		Homozygous (T/T)	32 (11)	1 (2)		
	<i>TLR1</i> SNPs Combined	<3 <i>TLR1</i> SNPs	291 (97)	39 (93)	Reference	0.09
3 <i>TLR1</i> SNPs		8 (3)	3 (7)	3.23 (0.82–12.79)		
<i>CD58</i>	rs17035850 ^b	Homozygous (A/A)	291 (97)	42 (100)	Reference	0.28
		Heterozygous (A/T)	8 (3)	0 (0)	0.40 (0.02–7.12) ^c	
	rs12025416	Homozygous (C/C)	229 (77)	28 (67)	Reference	0.17
		Heterozygous (C/T)	63 (21)	12 (28)	1.65 (0.81–3.39)	
<i>LCE4A-Clorf68</i>	rs4845320 ^b	Homozygous (A/A)	290 (97)	41 (98)	Reference	0.66
		Heterozygous (A/C)	9 (3)	1 (2)	0.61 (0.07–5.45)	
<i>TAGAP</i>	rs3127214	Homozygous (C/C)	282 (94)	40 (95)	Reference	0.36
		Heterozygous (C/T)	16 (5)	2 (5)	0.40 (0.06–2.85)	
		Homozygous (T/T)	1 (1)	0 (0)		

CLABSI, central line-associated bloodstream infection; SNP, single-nucleotide polymorphism.

^aOdds ratios were calculated using a dominant model (ie, heterozygote combined with the minor allele homozygote as risk genotypes). Patients with and without candidemia were compared using logistic regression analysis, after adjusting for non-*Candida*-related CLABSI rate.

^bNo patients with a homozygote minor allele genotype were observed.

^cEstimated, unadjusted odds ratio.

Association Between SNPs and Candidemia

Genotype distribution for *TLR1*, *CD58*, *LCE4A-Clorf68*, or *TAGAP* in patients with and without a candidemia are presented in Table 2. For all SNPs, the genotype distribution did not deviate from the Hardy-Weinberg equilibrium. In addition, the entire HPN patient population showed a similar allele distribution when compared with previously reported control groups.^{6,14}

In the logistic regression analysis, none of the SNPs were significantly associated with candidemia (Table 2). When adjusting for multiple candidemias per patient and the period of HPN dependency, similar results were observed (Table S2). Haploview analysis showed a strong linkage between all 3 *TLR1* SNPs (Figure 1). On patient level, there was a trend toward an increased risk for candidemia for the GAT haplotype for *TLR1* when compared with other haplotypes (OR 2.35; 95% CI, 0.87–6.34; $P = 0.09$). The patient *TLR1* diplotype distribution is shown in Table S3.

SNPs Associated With Severity of Infection

The severity of infection was recorded in 41 candidemia patients. In total, 7 (17%) and 15 (37%) patients had a persisting and/or disseminated candidemia during

follow-up, respectively. Genotype distribution for *TLR1*, *CD58*, *LCE4A-Clorf68*, and *TAGAP* are presented in Table 3. None of the SNPs were significantly associated with persisting candidemia. Similar results were observed for patients with or without a disseminated candidemia (Table 3).

Clinical Risk Factors for Candidemia

Univariable and multivariable Poisson regression analyses of clinical risk factors are shown in Table S4 and Table 4, respectively. Both intestinal dysmotility as underlying condition leading to IF, and the rate of non-*Candida*-related CLABSIs were univariably associated with candidemia. In a multivariable model, only a higher rate of non-*Candida*-related CLABSIs was associated with a higher risk for candidemia (rate ratio, 1.29; 95% CI, 1.14–1.46; $P < 0.001$).

Discussion

This is the first study to investigate host-genetic and clinical risk factors for candidemia in patients receiving HPN. We did not find any evidence for linkage of known risk SNPs in *TLR1*, *CD58*, *LCE4A-Clorf68*, or *TAGAP* and candidemia or, for that matter, the severity of infection. The *TLR1* GAT haplotype showed a trend toward an increased risk

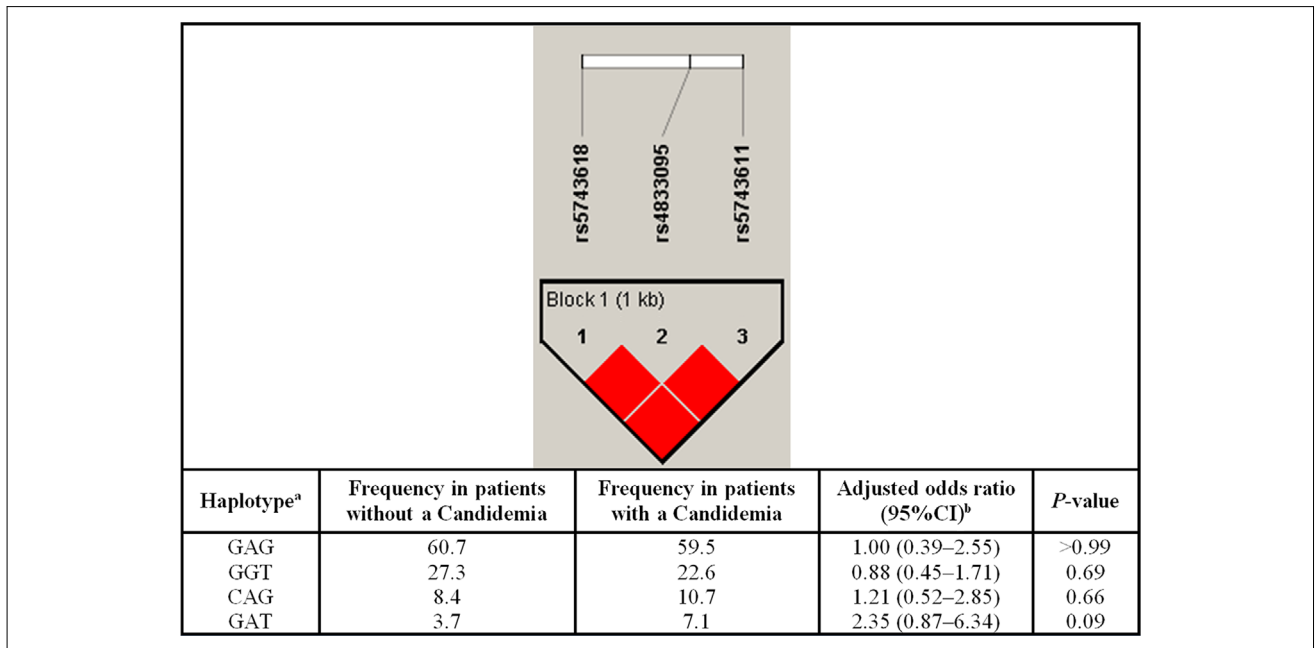


Figure 1. Linkage disequilibrium analysis and frequency distribution of haplotypes of *TLRI*. Pairwise linkage disequilibrium was assessed using D' . All *TLRI* SNPs (rs5743611, rs4833095, and rs5743618) were in linkage disequilibrium. Patient diplotype distributions are shown in Table S3. ^aSNPs are displayed in the following order: rs5743611, rs4833095, and rs5743618. ^bOn a patient level, haplotypes were compared vs all other haplotypes, using logistic regression analysis, after adjusting for non-*Candida*-related CLABSI rate. CLABSI; central-line associated bloodstream infection, SNP; single-nucleotide polymorphism.

for candidemia in patients receiving HPN. Not unexpected, the rate of non-*Candida*-related CLABSIs proved to be an independent clinical risk factor for candidemia.

In contrast to the studies by Plantinga and Kumar et al, we did not observe an increased susceptibility to candidemia in patients with SNPs in *TLRI*, *CD58*, *LCE4A*, or *TAGAP*. A reason for these seemingly conflicting findings may be that, although these SNPs have been previously identified in large candidemia cohorts, the results have not been replicated in other large candidemia cohorts yet. We cannot rule out that the investigated SNPs are not truly associated with candidemia. In addition, an important limitation is that the group size of candidemia patients in the present study was small. This clearly impacted on the power of our analyses, especially in some SNPs (eg, rs17035850, rs4845320, and rs3127214) with a low genotype frequency. Merely a limited power, however, cannot explain that certain highly frequent genotype SNPs (eg, rs4833095, or rs5743611 in the Poisson analysis) showed a trend toward a *decreased* risk for candidemia. This suggests that our HPN cohort differed from the patient populations investigated by Plantinga and Kumar et al. Indeed, these studies included candidemia patients with various clinical backgrounds. For example, a substantial number of patients had a compromised immune function (59%–64%), had active malignancy (32%–35%), used chemotherapy (16%–19%), or were admitted to the intensive care unit (49%–54%),

whereas only 19%–22% of patients used parenteral nutrition administered via a CVAD.^{6,14} It is unclear whether the latter subgroup had similar associations or trends between SNPs and candidemia. In contrast, our patient cohort had 1 major common risk factor for candidemia (ie, parenteral nutrition via a CVAD), whereas other major risk factors for candidemia were mostly lacking. Differences were also observed in 30-day mortality rates. In the previous studies, mortality rates of approximately 28% have been described, compared with 5% in our cohort.⁶ These high mortality rates are often reported in critically ill patients on intensive care units, whereas chronic IF patients tend to be more healthy and live in a relative stable condition at home.²³ Of note, it is unlikely that virulence of certain *Candida* species played a role, as the distribution of cultured species was similar to other studies.^{5,8,9}

Plantinga et al previously reported in healthy volunteers having 3 *TLRI* SNPs a significantly decreased expression of IL-1 β , IL-6, and IL-8. For this reason, we combined all 3 *TLRI* SNPs in our analysis. It was interesting that there was a trend toward an increased susceptibility to candidemia in patients with a *TLRI* GAT haplotype (7% vs 14%). Remarkably, in contrast to the study from Plantinga et al, we did not observe any patient with a CGT haplotype in our cohort. It is likely that the previously mentioned healthy volunteers actually had a CAG/GGT diplotype. Another explanation for this discrepancy is that the genotype

Table 3. Genetic Distribution of Patients With and Without a Persisting Candidemia or Disseminated Candidemia.^a

Gene/ Locus	SNP	Genotype	No Persisting Candidemia (%) (n = 34)	Persisting Candidemia (%) (n = 7)	Adjusted Odds Ratio (95% CI) ^b	P-Value	No Disseminated Candidemia (%) (n = 26)	Disseminated Candidemia (%) (n = 15)	Adjusted Odds Ratio (95% CI) ^b	P-Value
<i>TLRI</i>	rs5743611	Homozygous (G/G)	27 (79)	6 (86)	Reference	0.81	22 (85)	11 (73)	Reference	0.43
		Heterozygous (G/C)	6 (18)	1 (14)	0.75 (0.08–7.51)		4 (15)	3 (20)	1.90 (0.39–9.37)	
	rs4833095	Homozygous (C/C)	1 (3)	0 (0)	Reference	0.38	0 (0)	1 (7)	Reference	0.28
		Heterozygous (A/A)	20 (59)	3 (43)	2.17 (0.39–12.14)		13 (50)	10 (67)	0.47 (0.12–1.38)	
rs5743618	Heterozygous (A/G)	13 (38)	4 (57)	Reference	0.82	12 (46)	5 (33)	Reference	0.74	
	Homozygous (G/G)	1 (3)	0 (0)	1.22 (0.22–6.84)		1 (4)	0 (0)	0.809 (0.21–2.97)		
	Homozygous (G/G)	15 (44)	3 (43)	Reference	0.67	11 (42)	7 (47)	Reference	0.17	
<i>CD58</i>	TLRI SNPs Combined	Heterozygous (G/T)	18 (53)	4 (57)	0.60 (0.03–12.90) ^c	>0.99	14 (54)	8 (53)	0.22 (0.01–4.49) ^c	>0.99
		Homozygous (T/T)	1 (3)	0 (0)	Reference	0.74	1 (4)	0 (0)	Reference	>0.99
	3 <i>TLRI</i> SNPs	31 (91)	7 (100)	4.60 (0.08–250.82) ^c		23 (88)	15 (100)	1.71 (0.03–90.56) ^c		
<i>LCE4A- Clorf68</i>	rs17035850 ^d	Homozygous (A/A)	3 (9)	0 (0)	Reference	0.65	3 (12)	0 (0)	Reference	0.44
		Heterozygous (A/T)	34 (100)	7 (100)	1.34 (0.24–7.63)		26 (100)	15 (100)	1.00 (0.25–3.96)	
	rs12025416	Homozygous (C/C)	23 (68)	4 (57)	Reference	0.70	17 (65)	10 (67)	Reference	0.27
<i>TAGAP</i>	rs4845320 ^d	Heterozygous (C/T)	10 (29)	2 (29)	1.49 (0.06–40.25) ^c	0.70	9 (35)	3 (20)	0.55 (0.02–14.32) ^c	
		Homozygous (T/T)	1 (3)	1 (14)	Reference	0.70	0 (0)	2 (13)	Reference	
	rs3127214 ^d	Heterozygous (A/A)	33 (97)	7 (100)	2.15 (0.04–105.00)		25 (96)	15 (100)	0.32 (0.01–7.03) ^c	
		Heterozygous (A/C)	1 (3)	0 (0)			1 (4)	0 (0)		
		Homozygous (C/C)	33 (97)	6 (86)			24 (92)	15 (100)		
		Heterozygous (C/T)	1 (3)	1 (14)			2 (8)	0 (0)		

CLABSI, central line-associated bloodstream infection; SNP, single-nucleotide polymorphism.

^aIn 1 patient, data of persisting and disseminated candidemia was missing.

^bOdds ratios were calculated using a dominant model (ie, heterozygote combined with the minor allele homozygote as risk genotypes). Patients with and without a persisting candidemia were compared using logistic regression analysis, after adjusting for non-*Candida*-related CLABSI rate.

^cEstimated, unadjusted odds ratio.

^dNo patients with a homozygote minor allele genotype were observed.

Table 4. Multivariable Poisson Regression Analysis of Factors Associated With Candidemia.

Dependent Variable	Independent Variables	Rate Ratio (95% CI)	P-Value
Multivariable Poisson regression analysis			
Candidemia	Underlying disease		
	Short bowel syndrome	Reference	0.15
	Gastrointestinal motility disorder	2.02 (0.77–5.31)	0.33
	Mechanical obstruction	2.98 (0.33–26.98)	0.13
	Small bowel mucosal disease	4.31 (0.66–26.84)	0.72
	Intestinal fistula	1.69 (0.10–28.61)	
	Motility disorder	Reference	
	Mechanical obstruction	1.47 (0.16–13.49)	0.73
	Small bowel mucosal disease	2.08 (0.32–13.47)	0.44
	Intestinal fistula	0.83 (0.05–14.14)	0.90
	Mechanical obstruction	Reference	
	Small bowel mucosal disease	1.41 (0.09–21.38)	0.80
	Intestinal fistula	0.57 (0.02–18.13)	0.75
	Small bowel mucosal disease	Reference	
	Intestinal fistula	0.40 (0.02–10.43)	0.58
Non- <i>Candida</i> -related CLABSI (rate) ^a	1.29 (1.14–1.46)	<0.001	

Risk factors with a *P*-value of ≤ 0.2 in the univariable Poisson regression analysis (Table S4) were included in the final multivariable Poisson regression analysis.

CLABSI, central line-associated bloodstream infection.

^aNon-*Candida*-related CLABSI rate is expressed as the number of bloodstream infections per 1000 catheter days.

distribution of rs5743611 significantly deviated from the Hardy-Weinberg equilibrium in the study from Plantinga et al, possibly because of the different genotyping method that was used by these authors (mass spectrometry instead of PCR analysis).

In the present study, none of the investigated SNPs were significantly associated with persisting or disseminated disease. This is in contrast to previous studies, which linked rs17035850 in *CD58* and rs3127214 in *TAGAP* to persisting or disseminated candidemia, respectively.^{6,14} Differences in clinical setting may be a reason for these contradictory findings. Other genetic factors have been related to the severity of disease as well. For example, Choi et al identified 3 SNPs in *IL-4* related to chronic disseminated candidiasis, whereas Johnson et al showed that SNPs in *IL-10* and *IL-12B* were associated with persisting candidemia.^{24,25}

It may well be that in patients receiving HPN, risk factors other than genetic variation play a more important role in the development of candidemia. Our multivariable Poisson regression analysis showed a clear association between the rate of non-*Candida*-related CLABSIs and candidemia. We included non-*Candida*-related CLABSI rate as a surrogate marker for patient or caregiver adherence to aseptic catheter-handling protocols, as it remains very difficult to qualify such care in patients. Not unexpected, patients with a higher non-*Candida*-related CLABSI rate experienced candidemia more often, suggesting that the quality of aseptic catheter handling per se is relevant for *Candida* susceptibility. This observation again underpins the importance of strict adherence to aseptic protocols in HPN

care. It is, however, also important to note that the use of antibiotics may have been increased in patients experiencing non-*Candida*-related CLABSIs, which could subsequently have led to opportunistic *Candida* infections.²⁶

A strength of this study is that it concerns the largest case vs disease-matched control study in patients receiving HPN. In addition, we investigated all currently known genetic risk factors for candidemia to date. In previous studies, SNPs in *TLR2* and *TLR4* have been linked to candidemia as well. However, these results were not replicated in larger candidemia cohorts and therefore were excluded from this study.^{6,13,27} Finally, in contrast to other studies, we adjusted for multiple *Candida* events and exposure time to HPN in a secondary Poisson regression analysis, which might give a more accurate assessment of risk factors.^{6,13,14,27}

As mentioned earlier, a clear limitation is that only a small group of patients experienced candidemia, which impacted on the power of this study. To our knowledge, we identified all candidemia episodes in our patient cohort, but we cannot rule out that candidemia may have been missed in some patients. In addition, we were unable to include all previously reported relevant risk factors for candidemia, such as antibiotic use. Finally, we did not adjust for multiple comparisons in our analyses.

Future research should focus on establishing a larger, preferably prospective, international, multicenter HPN cohort to assess the impact of the investigated SNPs or the *TLR1* GAT haplotype and candidemia. Other SNPs may be validated as well, such as the previously mentioned SNPs in *IL-4*, *IL-10* and *IL-12B*.^{24,25} This could eventually help

identify patients at risk for candidemia and guide patient-specific preventive screening strategies.

In conclusion, in this study we could not replicate previously reported associations between SNPs in *TLR1*, *CD58*, *LCE4A-Clorf68*, or *TAGAP* and candidemia, or the severity of infection, in our HPN patient cohort. A possible role of the *TLR1* GAT haplotype in patients with candidemia has yet to be resolved. The identification of non-*Candida*-related CLABSI rate as a clinical risk factor for candidemia emphasizes the importance of catheter care to prevent candidemia in patients receiving HPN.

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Statement of Authorship

Y. Wouters, H. M. J. Roelofs, M. G. Netea, R. H. M. te Morsche, and G. J. A. Wanten equally contributed to the conception and design of the research; Y. Wouters, H. M. J. Roelofs, and R. H. M. te Morsche contributed to the acquisition and analysis of the data; Y. Wouters, H. M. J. Roelofs, M. G. Netea, R. H. M. te Morsche, and G. J. A. Wanten contributed to the interpretation of the data. All authors drafted the manuscript, critically revised the manuscript, agree to be fully accountable for ensuring the integrity and accuracy of the work, and read and approved the final manuscript

Supplementary Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

References

- Dreesen M, Foulon V, Spriet I, et al. Epidemiology of catheter-related infections in adult patients receiving home parenteral nutrition: A systematic review. *Clin Nutr*. 2013;32(1):16-26.
- Tribler S, Brandt CF, Fuglsang KA, et al. Catheter-related bloodstream infections in patients with intestinal failure receiving home parenteral support: risks related to a catheter-salvage strategy. *Am J Clin Nutr*. 2018;107(5):743-753.
- Wouters Y, Roosenboom B, Causevic E, Kievit W, Groenewoud H, Wanten GJA. Clinical outcomes of home parenteral nutrition patients using taurolidine as catheter lock: a long-term cohort study. *Clin Nutr*. 2018; pii: S0261-5614(18)32458-0.
- Wisplinghoff H, Bischoff T, Tallent SM, Seifert H, Wenzel RP, Edmond MB. Nosocomial bloodstream infections in US hospitals: analysis of 24,179 cases from a prospective nationwide surveillance study. *Clin Infect Dis*. 2004;39(3):309-317.
- Tortorano AM, Peman J, Bernhardt H, et al. Epidemiology of candidaemia in Europe: results of 28-month European Confederation of Medical Mycology (ECMM) hospital-based surveillance study. *Eur J Clin Microbiol Infect Dis*. 2004;23(4):317-322.
- Plantinga TS, Johnson MD, Scott WK, et al. Toll-like receptor 1 polymorphisms increase susceptibility to candidemia. *J Infect Dis*. 2012;205(6):934-943.
- Smekens SP, van de Veerdonk FL, Kullberg BJ, Netea MG. Genetic susceptibility to *Candida* infections. *EMBO Mol Med*. 2013;5(6):805-813.
- Wisplinghoff H, Ebbers J, Geurtz L, et al. Nosocomial bloodstream infections due to *Candida* spp. in the USA: species distribution, clinical features and antifungal susceptibilities. *Int J Antimicrob Agents*. 2014;43(1):78-81.
- Kullberg BJ, Arendrup MC. Invasive candidiasis. *N Engl J Med*. 2015;373(15):1445-1456.
- Ziegler MJ, Pellegrini DC, Safdar N. Attributable mortality of central line associated bloodstream infection: systematic review and meta-analysis. *Infection*. 2015;43(1):29-36.
- Brandt CF, Tribler S, Hvistendahl M, et al. Home parenteral nutrition in adult patients with chronic intestinal failure: catheter-related complications over 4 decades at the main Danish tertiary referral center. *JPEN J Parenter Enteral Nutr*. 2018;42(1):95-103.
- Pironi L, Arends J, Bozzetti F, et al. ESPEN guidelines on chronic intestinal failure in adults. *Clin Nutr*. 2016;35(2):247-307.
- Van der Graaf CA, Netea MG, Morre SA, et al. Toll-like receptor 4 Asp299Gly/Thr399Ile polymorphisms are a risk factor for *Candida* bloodstream infection. *Eur Cytokine Netw*. 2006;17(1):29-34.
- Kumar V, Cheng SC, Johnson MD, et al. Immunochip SNP array identifies novel genetic variants conferring susceptibility to candidaemia. *Nat Commun*. 2014;5:4675.
- Jackson B, Tilli CM, Hardman MJ, et al. Late cornified envelope family in differentiating epithelia—response to calcium and ultraviolet irradiation. *J Invest Dermatol*. 2005;124(5):1062-1070.
- Mao M, Biery MC, Kobayashi SV, et al. T lymphocyte activation gene identification by coregulated expression on DNA microarrays. *Genomics*. 2004;83(6):989-999.
- Versleijen MW, Huisman-de Waal GJ, Kock MC, et al. Arteriovenous fistulae as an alternative to central venous catheters for delivery of long-term home parenteral nutrition. *Gastroenterology*. 2009;136(5):1577-1584.
- Wouters Y, Vissers RK, Groenewoud H, Kievit W, Wanten GJA. Repair of damaged central venous catheters is safe and doubles catheter survival: a home parenteral nutrition patient cohort study. *Clin Nutr*. 2018; pii: S0261-5614(18)31347-5.
- Liang SY, Marschall J. Update on emerging infections: news from the Centers for Disease Control and Prevention. Vital signs: central line-associated blood stream infections—United States, 2001, 2008, and 2009. *Ann Emerg Med*. 2011;58(5):447-451.
- Prevention CfDca. Bloodstream Infection Event (Central Line-Associated Bloodstream Infection and Non-central Line Associated Bloodstream Infection). <https://www.cdc.gov/nhsn/acute-care-hospital/clabsi/index.html>. Reviewed December 14, 2018.
- SNPCheck version 3 Available from: <https://genetools.org/SNPCheck/snpcheck.htm>.
- Gabriel SB, Schaffner SF, Nguyen H, et al. The structure of haplotype blocks in the human genome. *Science*. 2002;296(5576):2225-2229.
- Lausch KR, Sogaard M, Rosenvinge FS, et al. High incidence of candidaemia in a nationwide cohort: underlying diseases, risk factors and mortality. *Int J Infect Dis*. 2018;76:58-63.
- Choi EH, Foster CB, Taylor JG, et al. Association between chronic disseminated candidiasis in adult acute leukemia and common IL4 promoter haplotypes. *J Infect Dis*. 2003;187(7):1153-1156.
- Johnson MD, Plantinga TS, van de Vosse E, et al. Cytokine gene polymorphisms and the outcome of invasive candidiasis: a prospective cohort study. *Clin Infect Dis*. 2012;54(4):502-510.
- Das I, Nightingale P, Patel M, Jumaa P. Epidemiology, clinical characteristics, and outcome of candidemia: experience in a tertiary referral center in the UK. *Int J Infect Dis*. 2011;15(11):e759-e763.
- Woehrl T, Du W, Goetz A, et al. Pathogen specific cytokine release reveals an effect of TLR2 Arg753Gln during *Candida* sepsis in humans. *Cytokine*. 2008;41(3):322-329.