

# Polymorphisms in Receptors Involved in Opsonic and Nonopsonic Phagocytosis, and Correlation with Risk of Infection in Oncohematology Patients

M. Carmen Herrero-Sánchez,<sup>a</sup> Eduardo B. Angomás,<sup>b</sup> Cristina de Ramón,<sup>c</sup> Juan J. Tellería,<sup>a,d</sup> Luis A. Corchete,<sup>d,e,f</sup> Sara Alonso,<sup>a</sup> M. del Carmen Ramos,<sup>g</sup> María J. Peñarrubia,<sup>c</sup> Saioa Márquez,<sup>h</sup> Nieves Fernández,<sup>h</sup> Luis J. García Frade,<sup>b</sup> Mariano Sánchez Crespo<sup>a</sup>

<sup>a</sup>Instituto de Biología y Genética Molecular, CSIC-Universidad de Valladolid, Valladolid, Spain

<sup>b</sup>Servicio de Hematología, Hospital Universitario Río-Hortega, Valladolid, Spain

<sup>c</sup>Servicio de Hematología, Hospital Clínico Universitario de Valladolid, Valladolid, Spain

<sup>d</sup>Instituto de Investigación Biomédica de Salamanca, Salamanca, Spain

<sup>e</sup>Departamento de Hematología, Hospital Universitario de Salamanca, Salamanca, Spain

<sup>f</sup>Centro de Investigación del Cáncer, Universidad de Salamanca, Salamanca, Spain

<sup>g</sup>Servicio de Microbiología, Hospital Universitario Río-Hortega, Valladolid, Spain

<sup>h</sup>Departamento de Bioquímica y Biología Molecular, Facultad de Medicina, Universidad de Valladolid, Valladolid, Spain

**ABSTRACT** High-risk hematological malignancies are a privileged setting for infection by opportunistic microbes, with invasive mycosis being one of the most serious complications. Recently, genetic background has emerged as an unanticipated risk factor. For this reason, polymorphisms for genes encoding archetypal receptors involved in the opsonic and nonopsonic clearance of microbes, pentraxin-3 (PTX3) and Dectin-1, respectively, were studied and correlated with the risk of infection. Fungal, bacterial, and viral infections were registered for a group of 198 patients with high-risk hematological malignancies. Polymorphisms for the pentraxin-3 gene (*PTX3*) showed a significant association with the risk of fungal infection by *Candida* spp. and, especially, by *Aspergillus* spp. This link remained even for patients undergoing antifungal prophylaxis, thus demonstrating the clinical relevance of PTX3 in the defense against fungi. *CLEC7A* polymorphisms did not show any definite correlation with the risk of invasive mycosis, nor did they influence the expression of Dectin-1 isoforms generated by alternative splicing. The *PTX3* mRNA expression level was significantly lower in samples from healthy volunteers who showed these polymorphisms, although no differences were observed in the extents of induction elicited by bacterial lipopolysaccharide and heat-killed *Candida albicans*, thus suggesting that the expression of PTX3 at the start of infection may influence the clinical outcome. *PTX3* mRNA expression can be a good biomarker to establish proper antifungal prophylaxis in immunodepressed patients.

**KEYWORDS** fungal infection, PTX3, SNP, Dectin-1, oncohematology

**M**anagement of opportunistic infections in patients with hematological malignancies is a difficult task. Immunosuppression due to the progress of the disease and the risks associated with chemotherapy and hematopoietic stem cell transplantation (HSCT) pave the way for microbial invasion. Despite the use of antifungal therapy, infection by *Candida* spp. and *Aspergillus* spp. remains an important cause of mortality (1, 2). In addition, several studies have confirmed the existence of risk factors associated with genetic polymorphisms in the immune system (3–7). In this regard, polymorphisms in genes coding for components of innate immunity, such as the pattern

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Address correspondence to Mariano Sánchez Crespo, [mrcrespo@ibgm.uva.es](mailto:mrcrespo@ibgm.uva.es).

recognition receptors (PRRs) Dectin-1 and long pentraxin 3 (PTX3), have been identified as risk factors in oncohematology patients (3, 8–11).

Dectin-1 is a C-type lectin transmembrane receptor encoded by the *CLEC7A* gene that, through alternative splicing, generates various receptor isoforms, with the A and B isoforms being the only functional ones (12). Both isoforms recognize the most abundant fungal cell wall polysaccharides, i.e., the  $\beta(1\rightarrow3)$ - and/or  $\beta(1\rightarrow6)$ -linked glucans, trigger nonopsonic phagocytosis, and initiate a proinflammatory response (13). Among the several *CLEC7A* polymorphisms studied in oncohematology patients, rs16910526 has been associated with risks of both candidiasis and aspergillosis. This single nucleotide polymorphism (SNP) entails a loss of function of the protein by generating an early stop codon leading to the loss of amino acids in the C-terminal domain, which affects  $\beta$ -glucan recognition (14). The presence of  $\beta$ -glucans on some bacteria entails their recognition by Dectin-1. Nonetheless, mycobacteria, which do not express  $\beta$ -glucans, may also be recognized (15, 16). In addition, rs2078178 and rs16910631 polymorphisms have been associated with ulcerative colitis (17) and Asperger disease (18), i.e., diseases associated with immune dysfunction in the gastrointestinal tract.

PTX3 is a soluble receptor encoded as a single transcript by the *PTX3* gene, which participates in opsonic phagocytosis by interacting with both complement components and Fc $\gamma$  receptors (Fc $\gamma$ Rs). Both *in vitro* and *in vivo* studies have shown the direct binding of PTX3 to the surface of fungi, bacteria, and viruses (19, 20). Genetic variants of *PTX3* in oncohematology patients have been studied previously (10, 11), and *PTX3* SNPs rs2305619, rs3816527, and rs1840680 have been associated with an increased risk of invasive mycosis (particularly aspergillosis). However, this association was not observed in either non-HSCT patients (10) or oncohematology patients with preexistent neutropenia (11). These polymorphisms have also shown a strong association with the risks of tuberculosis and colonization by *Pseudomonas* spp. (21, 22), which highlights an overall role of PTX3 in the innate immune response.

Given that both opsonic and nonopsonic phagocytoses are involved in host defense, we have selected model receptors for each mechanism of phagocytosis to disclose the role of their polymorphisms in the risk of infection in a series of patients with hematological malignancies. We selected polymorphisms rs16910526, rs2078178, and rs16910631 for *CLEC7A* and rs3816527, rs2305619, and rs1840680 for *PTX3*. Genotyping studies showed a marked association between *PTX3* SNPs and the risk of fungal infections. Irrespective of the *PTX3* genotype, the extent of *PTX3* mRNA expression correlated with susceptibility to infection, thus suggesting that a high level of *PTX3* expression is a key factor for an efficient innate immune response to fungal infection.

## RESULTS

**Patients.** A retrospective study was carried out with 198 oncohematology patients, whose clinical characteristics are shown in Table 1. The most frequent clinical diagnoses were acute myeloblastic leukemia (33.8%), lymphoma (31.3%), and acute lymphoblastic leukemia (10.6%). Approximately 41% of patients underwent HSCT: 58% underwent autologous and 42% underwent allogeneic HSCT. Table 2 shows the percentages of patients affected by different microorganisms (78.8%): bacteria were most frequently detected (66.7%), followed by fungal infections (46.5%) and, to a lesser extent, viral infections (17.7%). Diagnosis of fungal infection was carried out according to revised EORTC/MSG criteria (39). *Candida* species infections were the most common (38.4% of the total number of patients), 14.5% of which were considered invasive because of positive cultures in samples from at least two different tissues. The remainder showed positive cultures for *Candida* in mucosae, urine, and stool, which were considered evidence of colonization. *Aspergillus* species infections were detected in 9.6% of the patients, 63.2% of which were considered invasive according to EORTC/MSG criteria. Yeasts such as *Saccharomyces cerevisiae*, *Geotrichum candidum*, and *Rhodotorula mucilaginosa* were identified in a few patients.

**TABLE 1** Demographic and clinical characteristics

Parameter <sup>a</sup>	Value
Demographic variables	
No. of male patients/no. of female patients (% of male patients/% of female patients)	117/81 (59.1/40.9)
Median age (yr) (range)	62 (4–92)
No. (%) of patients with hematological malignancy	
AML	67 (33.8)
Lymphoma	62 (31.3)
ALL	21 (10.6)
MM	20 (10.1)
MDS	17 (8.6)
CLL	8 (4)
Other	3 (1.5)
No. (%) of patients with HSCT	
Allogenic	34 (42)
Autologous	47 (58)

<sup>a</sup>Abbreviations: AML, acute myeloblastic leukemia; ALL, acute lymphoblastic leukemia; MM, multiple myeloma; MDS, myelodysplastic syndrome; CLL, chronic lymphocytic leukemia; HSCT, hematopoietic stem cell transplantation.

**SNPs and risk of infection.** The association between the different SNPs and the risk of both colonization and infection was adjusted for sex, age, and hematological malignancy. After confirming that all SNPs were in Hardy-Weinberg equilibrium (HWE), at a *P* value of >0.05, within each group studied, none of the studied SNPs displayed any association with the risk of either bacterial or viral infections. In contrast, there was a significant association between *PTX3* SNP rs3816527 and the presence of *Candida* and *Aspergillus* spp. (Table 3). The calculation of odds ratio (OR) values, according to genotype distribution, revealed that the C allele of rs3816527 (on the basis of the best model fit [recessive model]) behaved as a risk factor ( $OR_{CC+CA} = 2.29$  [95% confidence interval {CI}, 1.20 to 4.36] [*P* value of 0.01]). However, when the incidences of fungal colonization and invasive disease (36.34% and 11.1%, respectively) were evaluated separately, the analysis exhibited a significant association with invasive mycosis only. As shown in Table 4, the AA genotype for intronic SNPs (rs2305619 and rs1840680) displayed a significant risk ( $OR_{rs2305619AA} = 3.28$  [95% CI, 1.24 to 8.69] [*P* value of 0.02];  $OR_{rs1840680AA} = 3.98$  [95% CI, 1.52 to 10.4] [*P* value of 0.0058]). Although the analysis was not significant for rs3816527, the CC genotype exhibited a certain degree of

**TABLE 2** Percentages of patients affected by pathogens, the recognition of which has been associated with Dectin-1 and *PTX3*

Pathogen	No (%) of patients affected <sup>a</sup>
Bacteria	
Genus <i>Pseudomonas</i>	132 (66.7)
<i>Klebsiella pneumoniae</i>	29 (14.6)
<i>Staphylococcus aureus</i>	15 (7.6)
Genus <i>Staphylococcus</i>	61 (30.8)
<i>Staphylococcus aureus</i>	16 (8.1)
<i>Mycobacterium tuberculosis</i>	2 (1)
Fungi	
Genus <i>Candida</i>	92 (46.5)
Colonization	76 (38.4)
Invasive disease	65 (32.8; 85.5*)
Genus <i>Aspergillus</i>	11 (5.6; 14.5*)
Colonization	19 (9.6)
Invasive disease	7 (3.54; 36.8*)
Other yeasts	12 (6.1; 63.2*)
Other yeasts	10 (5.1)
Viruses	
Cytomegalovirus	35 (17.7)
Cytomegalovirus	12 (6.1)

<sup>a</sup>Percentages were calculated based on the total number of patients, except for the values marked with an asterisk, which refer to the number of patients infected with the respective pathogen.

**TABLE 3** Genotype distribution of *PTX3* SNPs in patients with and without detection of *Candida* spp. and/or *Aspergillus* spp. and association thereof<sup>a</sup>

SNP	Model	Genotype	No. (%) of patients with <i>Candida</i> or <i>Aspergillus</i> species detection	No. (%) of patients without <i>Candida</i> or <i>Aspergillus</i> species detection	OR (95% CI)	P value	BIC
rs2305619	Codominant	AA	23 (25.8)	18 (16.5)	1.00	0.18	318.6
		AG	46 (51.7)	58 (53.2)	1.65 (0.77–3.52)		
		GG	20 (22.5)	33 (30.3)	2.22 (0.94–5.24)		
	Dominant	AA	23 (25.8)	18 (16.5)	1.00	0.099	314.1
		AG+GG	66 (74.2)	91 (83.5)	1.83 (0.89–3.76)		
	Recessive	AA+AG	69 (77.5)	76 (69.7)	1.00	0.19	315
GG		20 (22.5)	33 (30.3)	1.55 (0.8–3.01)			
rs3816527	Codominant	CC	19 (21.4)	15 (13.8)	1.00	0.069	316.7
		CA	49 (55.1)	52 (47.7)	1.37 (0.6–3.1)		
		AA	21 (23.6)	42 (38.5)	2.56 (1.05–6.2)		
	Dominant	CC	19 (21.4)	15 (13.8)	1.00	0.16	314.8
		CA+AA	70 (78.7)	94 (86.2)	1.74 (0.8–3.78)		
	Recessive	<b>CC+CA</b>	<b>68 (76.4)</b>	<b>67 (61.5)</b>	<b>1.00</b>	<b>0.029</b>	<b>312</b>
<b>AA</b>		<b>21 (23.6)</b>	<b>42 (38.5)</b>	<b>2.02 (1.07–3.84)</b>			
rs1840680	Codominant	AA	24 (27)	18 (16.5)	1.00	0.092	317.3
		AG	45 (50.6)	55 (50.5)	1.68 (0.79–3.57)		
		GG	20 (22.5)	36 (33)	2.54 (1.09–5.94)		
	Dominant	AA	24 (27)	18 (16.5)	1.00	0.066	313.4
		AG+GG	65 (73)	91 (83.5)	1.94 (0.95–3.98)		
	Recessive	AA+AG	69 (77.5)	73 (67)	1.00	0.086	313.8
GG		20 (22.5)	36 (33)	1.76 (0.92–1.76)			

<sup>a</sup>Model adjusted for sex, age, and hematological malignancy. For 198 patients, the SNPStats program established the most frequent genotype as the default reference and different inheritance models. The best-fit model is indicated in boldface type, as are the significant associations at a *P* value of <0.05. Abbreviations: OR, odds ratio; CI, confidence interval; BIC, Bayesian information criterion.

association (OR<sub>rs3816527CC</sub> = 2.55 [95% CI, 0.9 to 7.16] [*P* value of 0.086]) (Table 4). Moreover, the risk of invasive aspergillosis showed a robust association for three *PTX3* SNPs (OR<sub>rs2305619AA</sub> = 4.2 [95% CI, 1.22 to 14.57] [*P* value of 0.026]; OR<sub>rs3816527CC</sub> = 5.1 [95% CI, 1.45 to 17.97] [*P* value of 0.013]; OR<sub>rs1840680AA</sub> = 4.15 [95% CI, 1.21 to 14.3] [*P* value of 0.027]) (Table 5). *PTX3* also exhibited a significant correlation with the risk of

**TABLE 4** Genetic association of *PTX3* SNPs with invasive fungal infection<sup>a</sup>

SNP	Model	Genotype	No. (%) of patients with IFI	No. (%) of patients without IFI	OR (95% CI)	P value	BIC
rs2305619	Codominant	AA	9 (40.9)	32 (18.2)	1.00	0.059	184.5
		AG	9 (40.9)	95 (54)	2.97 (1.04–8.4)		
		GG	4 (18.2)	49 (27.8)	4.04 (1.1–14.96)		
	Dominant	<b>AA</b>	<b>9 (40.9)</b>	<b>32 (18.2)</b>	<b>1.00</b>	<b>0.02</b>	<b>179.5</b>
		<b>AG+GG</b>	<b>13 (59.1)</b>	<b>144 (81.8)</b>	<b>3.28 (1.24–8.69)</b>		
	Recessive	AA+AG	18 (81.8)	127 (72.2)	1.00	0.21	183.3
GG		4 (18.2)	49 (27.8)	2.03 (0.63–6.50)			
rs3816527	Codominant	CC	7 (31.8)	27 (15.3)	1.00	0.17	186.7
		CA	10 (45.5)	91 (51.7)	2.17 (0.72–6.55)		
		AA	5 (22.7)	58 (33)	3.35 (0.9–12.16)		
	Dominant	CC	7 (31.8)	27 (15.3)	1.00	0.086	182
		CA+AA	15 (68.2)	149 (84.7)	2.55 (0.9–7.16)		
	Recessive	CC+CA	17 (77.3)	118 (67)	1.00	0.2	183.3
AA		5 (22.7)	58 (33)	1.97 (0.67–5.8)			
rs1840680	Codominant	AA	10 (45.5)	32 (18.2)	1.00	0.02	182.4
		AG	8 (36.4)	92 (52.3)	3.58 (1.26–10.2)		
		GG	4 (18.2)	52 (29.6)	4.87 (1.33–17.8)		
	Dominant	<b>AA</b>	<b>10 (45.5)</b>	<b>32 (18.2)</b>	<b>1.00</b>	<b>0.0058</b>	<b>177.3</b>
		<b>AG+GG</b>	<b>12 (54.5)</b>	<b>144 (81.8)</b>	<b>3.98 (1.52–10.4)</b>		
	Recessive	AA+AG	18 (81.8)	124 (70.5)	1.00	0.15	182.8
GG		4 (18.2)	52 (29.6)	2.24 (0.7–7.2)			

<sup>a</sup>Association analysis for 198 patients was adjusted for sex, age, and hematological malignancy. The SNPStats program established the dominant model as the best-fit model, which is indicated in boldface type, as are its significant associations at a *P* value of <0.05. IFI, invasive fungal infection.

**TABLE 5** Genotype association of *PTX3* SNPs with the risk of developing invasive aspergillosis<sup>a</sup>

SNP	Model	Genotype	No. (%) of patients with IA	No. (%) of patients without IA	OR (95% CI)	P value	BIC
rs2305619	Codominant	AA	6 (50)	35 (18.8)	1.00	0.069	134
		AG	3 (25)	101 (54.3)	5.3 (1.21–23.26)		
		GG	3 (25)	50 (26.9)	3.1 (0.68–14.14)		
	<b>Dominant</b>	<b>AA</b>	<b>6 (50)</b>	<b>35 (18.8)</b>	<b>1.00</b>	<b>0.026</b>	<b>129.1</b>
		<b>AG+GG</b>	<b>6 (50)</b>	<b>151 (81.2)</b>	<b>4.2 (1.22–14.57)</b>		
	Recessive	AA+AG	9 (75)	136 (73.1)	1.00	0.73	133.9
GG		3 (25)	50 (26.9)	1.27 (0.32–5.13)			
rs3816527	Codominant	CC	6 (50)	28 (15.1)	1.00	0.044	133.1
		CA	3 (25)	98 (52.7)	5.88 (1.3–26.27)		
		AA	3 (25)	60 (32.3)	4.3 (0.94–19.78)		
	<b>Dominant</b>	<b>CC</b>	<b>6 (50)</b>	<b>28 (15.1)</b>	<b>1.00</b>	<b>0.013</b>	<b>127.9</b>
		<b>CA+AA</b>	<b>6 (50)</b>	<b>158 (85)</b>	<b>5.1 (1.45–17.97)</b>		
	Recessive	CC+CA	9 (75)	126 (67.7)	1.00	0.48	133.5
AA		3 (25)	60 (32.3)	1.63 (0.41–6.52)			
rs1840680	Codominant	AA	6 (50)	36 (19.4)	1.00	0.076	134.2
		AG	3 (25)	97 (52.1)	5.06 (1.15–22.2)		
		GG	3 (25)	53 (28.5)	3.2 (0.7–14.62)		
	<b>Dominant</b>	<b>AA</b>	<b>6 (50)</b>	<b>36 (19.4)</b>	<b>1.00</b>	<b>0.027</b>	<b>129.2</b>
		<b>AG+GG</b>	<b>6 (50)</b>	<b>150 (80.7)</b>	<b>4.15 (1.21–14.3)</b>		
	Recessive	AA+AG	9 (75)	133 (71.5)	1.00	0.66	133.9
GG		3 (25)	53 (28.5)	1.36 (0.34–5.51)			

<sup>a</sup>Model adjusted for sex, age, and hematological malignancy for the 198 high-risk hematology patients. The dominant model was established as the best-fit model and is indicated in boldface type, as are its significant associations at a *P* value of <0.05. IA, invasive aspergillosis.

developing invasive candidiasis but only for SNP rs1840680 (OR = 3.8 [95% CI, 1.05 to 13.57] [*P* value of 0.048]) (Table 6). Antifungal prophylaxis (AP) is crucial for high-risk patients and is currently mandatory under these conditions (23). Nonetheless, despite AP with fluconazole, posaconazole, or micafungin, 11.43% of patients experienced invasive mycosis, whereas colonization was detected in 37.86% of patients (Table 7). A new analysis adjusted for the above-mentioned covariates plus AP showed similar results. New association analyses were performed to evaluate the risks of fungal

**TABLE 6** Influence of *PTX3* SNPs on the risk of invasive candidiasis in high-risk oncohematology patients<sup>a</sup>

SNP	Model	Genotype	No. (%) of patients with candidiasis	No. (%) of patients without candidiasis	OR (95% CI)	P value	BIC
rs2305619	Codominant	AA	4 (36.4)	37 (19.8)	1.00	0.18	137
		AG	6 (54.5)	98 (52.4)	1.94 (0.5–7.58)		
		GG	1 (9.1)	52 (27.8)	6.66 (0.69–64.0)		
	Dominant	AA	4 (36.4)	37 (19.8)	1.00	0.17	133.3
		AG+GG	7 (63.6)	150 (80.2)	2.62 (0.7–9.8)		
	Recessive	AA+AG	10 (90.9)	135 (72.2)	1.00	0.11	132.6
GG		1 (9.1)	52 (27.8)	4.26 (0.5–34.66)			
rs3816527	Codominant	CC	2 (18.2)	32 (17.1)	1.00	0.45	138.9
		CA	7 (63.6)	94 (50.3)	0.87 (0.16–4.65)		
		AA	2 (18.2)	61 (32.6)	2.3 (0.3–17.85)		
	Dominant	CC	2 (18.2)	32 (17.1)	1.00	0.83	135.1
		CA+AA	9 (81.8)	155 (82.9)	1.19 (0.24–6.04)		
	Recessive	CC+CA	9 (81.8)	126 (67.4)	1.00	0.21	133.6
AA		2 (18.2)	61 (32.6)	2.55 (0.52–12.5)			
rs1840680	Codominant	AA	5 (45.5)	37 (19.8)	1.00	0.07	135.1
		AG	5 (45.5)	95 (50.8)	2.75 (0.73–10.4)		
		GG	1 (9.1)	55 (29.4)	9.12 (0.99–84.3)		
	<b>Dominant</b>	<b>AA</b>	<b>5 (45.5)</b>	<b>37 (19.8)</b>	<b>1.00</b>	<b>0.048</b>	<b>131.3</b>
		<b>AG+GG</b>	<b>6 (54.5)</b>	<b>150 (80.2)</b>	<b>3.8 (1.05–13.57)</b>		
	Recessive	AA+AG	10 (90.9)	132 (70.6)	1.00	0.076	132
GG		1 (9.1)	55 (29.4)	4.8 (0.59–39.22)			

<sup>a</sup>Association analysis for the risk of invasive candidiasis in 198 patients. The study, adjusted for sex, age, and hematological malignancy, showed a significant association for SNP rs1840680. The significant associations at a *P* value of <0.05 are indicated in boldface type.

**TABLE 7** Influence of antifungal prophylaxis on the incidence of fungal colonization and systemic infection<sup>a</sup>

Parameter	No. of patients	% of patients
With antifungal prophylaxis (fluconazole, posaconazole, and/or micafungin)	140	70.7
Fungal colonization	53	37.86
Genus <i>Candida</i>	47	88.7
Genus <i>Aspergillus</i>	7	13.2
Invasive fungal infection	16	11.43
Genus <i>Candida</i>	9	56.25
Genus <i>Aspergillus</i>	8	50
Without antifungal prophylaxis	58	29.3
Fungal colonization	18	31.03
Genus <i>Candida</i>	18	100
Genus <i>Aspergillus</i>	0	0
Invasive fungal infection	6	10.34
Genus <i>Candida</i>	2	33.33
Genus <i>Aspergillus</i>	4	66.66

<sup>a</sup>The numbers of patients with and without antifungal prophylaxis and the respective percentages are represented, as are the incidences of acquired fungal colonization and invasive infection.

colonization and systemic infection in patients who received AP. *PTX3* SNPs displayed no association with the risk of fungal colonization. However, it is worth noting that there was a significant association of systemic aspergillosis for rs3816527 and a clear trend for intronic *PTX3* SNPs (OR<sub>rs3816527CC</sub> = 5.64 [95% CI, 1.18 to 26.88] [*P* value of 0.034]) (Table 8).

Regarding *CLEC7A* SNPs, a significant association with the different risk groups could not be observed. However, rs16910526 exhibited a certain degree of protective association in those patients presenting with *Candida* spp. and/or *Aspergillus* spp. (OR<sub>rs16910526TT</sub> = 2.28 [95% CI, 0.95 to 5.51] [*P* value of 0.058]) (see Table S1 in the supplemental material), particularly in those with fungal colonization (Table S2). Moreover, when the analysis was restricted to patients who received AP, the G allele for rs16910526 showed a significant protective effect versus the risk of fungal colonization (Table S3), particularly by *Candida* (Table S4). Although the relevance of this finding

**TABLE 8** Genetic susceptibility of *PTX3* SNPs to invasive aspergillosis in patients with antifungal prophylaxis<sup>a</sup>

SNP	Model	Genotype	No. (%) of patients with IA	No. (%) of patients without IA	OR (95% CI)	<i>P</i> value	BIC
rs2305619	Codominant	AA	4 (50)	28 (21.2)	1.00	0.19	101.1
		AG	3 (37.5)	67 (50.8)	3.25 (0.65–16.4)		
		GG	1 (12.5)	37 (28)	6.13 (0.6–62.33)		
	Dominant	AA	4 (50)	28 (21.2)	1.00	0.08	96.4
		AG+GG	4 (50)	104 (78.8)	3.94 (0.87–17.8)		
	Recessive	AA+AG	7 (87.5)	95 (72)	1.00	0.26	98.2
GG		1 (12.5)	37 (28)	3.1 (0.35–27.36)			
rs3816527	Codominant	CC	4 (50)	22 (16.7)	1.00	0.083	99.5
		CA	3 (37.5)	65 (49.2)	4.35 (0.81–23.3)		
		AA	1 (12.5)	45 (34.1)	9.55 (0.91–99.6)		
	<b>Dominant</b>	<b>CC</b>	<b>4 (50)</b>	<b>22 (16.7)</b>	<b>1.00</b>	<b>0.034</b>	<b>95</b>
		<b>CA+AA</b>	<b>4 (50)</b>	<b>110 (83.3)</b>	<b>5.64 (1.18–26.9)</b>		
	Recessive	CC+CA	7 (87.5)	87 (65.9)	1.00	0.16	97.5
AA		1 (12.5)	45 (34.1)	3.97 (0.44–35.6)			
rs1840680	Codominant	AA	4 (50)	29 (22)	1.00	0.18	101
		AG	3 (37.5)	63 (47.7)	3.14 (0.62–15.8)		
		GG	1 (12.5)	40 (30.3)	6.5 (0.64–67.25)		
	Dominant	AA	4 (50)	29 (22)	1.00	0.08	96.4
		AG+GG	4 (50)	103 (78)	3.94 (0.87–17.8)		
	Recessive	AA+AG	7 (87.5)	92 (69.7)	1.00	0.22	98
GG		1 (12.5)	40 (30.3)	3.4 (0.38–30.63)			

<sup>a</sup>For 140 patients undergoing antifungal prophylaxis, the analysis model was adjusted for sex, age, and hematological malignancy. The SNPStats program recognized the most frequent genotype as the default reference genotype and established the corresponding inheritance models. The best-fit model is indicated in boldface type, as are the significant associations at a *P* value of <0.05. IA, invasive aspergillosis.



**TABLE 9** Risk assessment of invasive aspergillosis for the haplotypes corresponding to rs2305619, rs3816527, and rs1840680<sup>a</sup>

Haplotype at rs2305619-rs3816527-rs1840680	Association risk for main haplotypes formed by PTX3 SNPs (frequency)					
	198 patients			140 patients with AP <sup>c</sup>		
	Total	IA <sup>b</sup>	No IA <sup>b</sup>	Total	IA	No IA
A-C-A	0.424	0.625	0.411	0.429	0.687	0.413
G-A-G	0.523	0.375	0.532	0.514	0.312	0.526

<sup>a</sup>Haplotype analyses were performed both on the overall population of 198 patients and on the 140 patients with AP. The assessment was adjusted for sex, age, hematological malignancy, and AP using the SNPStats program. Data represent the distributions of the two most frequent haplotypes and the association of the A-C-A haplotype with distinct infection groups. Associations at a *P* value of <0.05 are significant.

<sup>b</sup>OR, 2.95 (95% CI, 1.1 to 7.91) (*P* value of 0.032).

<sup>c</sup>OR, 3.12 (95% CI, 0.98 to 9.99) (*P* value of 0.057).

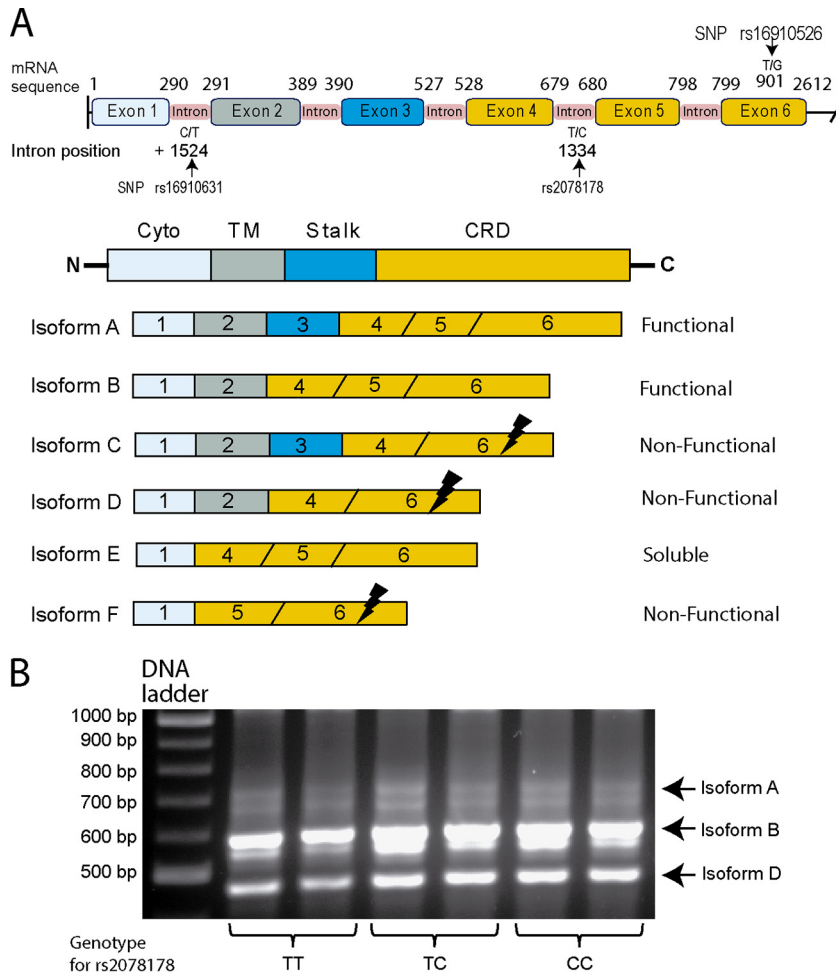
may be tempered by the low overall frequency of this polymorphism in patients with AP (GG, 1%; GT, 13%), it is noteworthy that neither of the two patients homozygous for rs16910526 had colonization. However, the only carrier of the GG genotype, who did not receive AP, experienced fungal colonization by *Candida* spp. (Table S5). Within the group of patients with AP, rs2078178 also showed a significant association with the risk of fungal colonization, although it did not exhibit any trend in other analysis groups (Table S6). In the case of rs16910631, the TT genotype was not observed, which explains why this polymorphism was not included for further analysis.

**Association of PTX3 SNP haplotypes with mycosis risk.** After confirming the strong association of PTX3 SNPs with the risk of invasive aspergillosis, their haplotype distribution was assessed, discarding possible confusion covariates. As shown in Table 9, the SNPStats program established that haplotype A-C-A (SNP positions rs2305619-rs3816527-rs1840680) was significantly associated with the risk of invasive aspergillosis, where 62.5% of carriers of this haplotype developed infection, versus 41.1% of patients who did not develop it (Table 9). Even in patients who received AP, this haplotype showed a strong trend toward association (Table 9).

**Meta-analysis for PTX3 SNPs.** In order to confirm the risk genotype, a meta-analysis was performed with the inclusion criteria described in Materials and Methods. Data from studies by Cunha et al. (10) and Brunel et al. (11) were selected for combined analysis with our data. As shown in Fig. S1 in the supplemental material, only the AA genotype for rs3816527 and the GG genotype for intronic SNPs were checked as risk factors for invasive aspergillosis. Meta-analysis displayed these genotypes as risk factors (OR<sub>rs2305619GG</sub> = 2.37 [95% CI, 1.23 to 4.57] [*P* value of 0.01]; OR<sub>rs3816527AA</sub> = 2.08 [95% CI, 1.01 to 4.28] [*P* value of 0.049]; OR<sub>rs1840680GG</sub> = 2.30 [95% CI, 1.14 to 4.61] [*P* value of 0.019]). In contrast, our data reflected a protector effect for these genotypes but with great uncertainty (OR<sub>rs2305619GG</sub> = 0.91 [95% CI, 0.24 to 3.48]; OR<sub>rs3816527AA</sub> = 0.70 [95% CI, 0.18 to 2.68]; OR<sub>rs1840680GG</sub> = 0.84 [95% CI, 0.22 to 3.21]). Although meta-analysis reflected a significant publication bias for SNP rs3816527 (Egger's *P* value of 0.0438), there was no clear evidence of publication bias for the other SNPs (Egger's *P* value of >0.05) (Fig. S2). Nevertheless, these genotypes did not show any significant effect in our study, as previously shown. On the other hand, our risk genotypes for this invasive mycosis could not be assessed by meta-analysis due to the lack of information in the studies by Cunha et al. and Brunel et al. regarding the number of individuals for the remainder of the genotypes.

**Prediction of SNP pathogenicity.** Given the significant association between SNP rs2078178 in *CLEC7A* and the risk of fungal colonization in the patients who received AP, the possible influence of this SNP on the alternative splicing of *CLEC7A* was assessed (Fig. 1A). *In silico* analysis did not show any impact on splicing by using the Human Splicing Finder program. Nevertheless, we sought the presence of the different transcripts of Dectin-1 in monocytes from healthy volunteer donors according to their genotypes. As shown in Fig. 1B, the two functional isoforms and the nonfunctional D isoform were expressed in monocytes independently of their genotype.

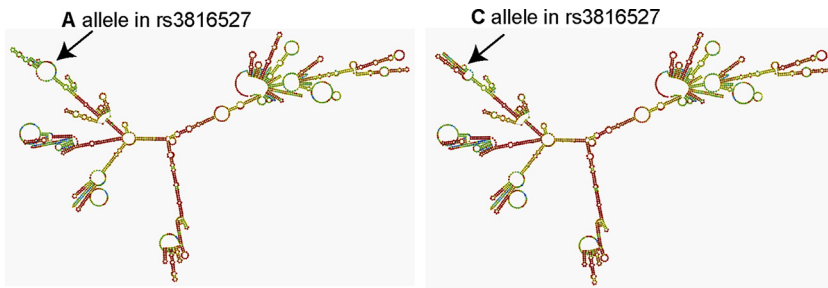
Regarding PTX3 SNPs, the change of nucleotide C for A in exonic SNP rs3816527 is responsible for the substitution of aspartic acid for alanine in the 48th position, the



**FIG 1** Genomic structure of Dectin-1, isoforms resulting from alternative splicing, and influence of the rs2078178 genotype. (A) The structures of *CLEC7A* and the different isoforms resulting from alternative splicing are shown together with the positions of the polymorphisms. The black lightning bolts indicate the positions of the stop codons introduced through frameshifts generated by alternative splicing. The diagram has been constructed based on the sequence under GenBank accession number NG\_016291.1 and data reported previously by Willment et al. (12). (B) Monocytes from buffy coats from healthy donors of the different rs2078178 genotypes were isolated and cultured for mRNA collection. The different isoforms of Dectin-1 were amplified by PCR and separated by electrophoresis on an agarose gel. Abbreviations: Cyto, cytoplasmic tail; TM, transmembrane region; CRD, carbohydrate recognition domain.

consequences of which were checked by the SIFT program. This program determined that both amino acids are tolerated at the 48th position (see Table S7 in the supplemental material). However, the RNAfold program showed that the exchange of both nucleotides could affect mRNA folding (Fig. 2). Unexpectedly, the folding pattern observed was somewhat different from the results reported by Cunha et al. (see Fig. S6 in reference 10). On this basis, the potential splice-site effects of SNP rs3816527 showed that allele C might create a new cryptic splicing site, which could compete against the canonical splicing site (Table 10). Consequently, the C allele would modify exon 2 expression and generate a smaller transcript (Fig. 3A). According to the primers used for amplifying *PTX3* mRNA in healthy donors, a normal transcript of 170 bp must be present in samples with the A allele, a 147-bp transcript must be present in samples with the C allele, and both transcripts must be present in the case of the CA genotype. However, independently of their genotype, all samples showed a band of 170 bp and some bands with higher mobility, even after stimulation (Fig. 3B). In the case of intronic *PTX3* SNPs (rs2305619 and rs1840680), the Human Splicing Finder program did not detect any type of impact on splicing (data not shown).





**FIG 2** Impact of SNP rs3816527 on the secondary structure of PTX3 mRNA. The effect of the exchange of nucleotides (A allele or C allele) is shown by the position of the arrows.

**PTX3 expression in monocytes.** To address possible functional consequences associated with the different polymorphisms, the expression of *PTX3* mRNA was assayed in monocytes under resting conditions and after stimulation with lipopolysaccharide (LPS) and heat-killed *Candida* conidia. As shown in Fig. 4A, the basal *PTX3* mRNA expression level was significantly lower in carriers of the rs3816527 CC genotype than in the CA+AA group. In the case of both intronic SNPs, the *PTX3* mRNA expression level was significantly lower in the carriers of AA genotype than in the AG+GG groups (Fig. 4A). In contrast, although *PTX3* mRNA expression was increased by the stimuli, no differences in expression were observed between genotypic groups of each SNP (Fig. 4B and C).

**DISCUSSION**

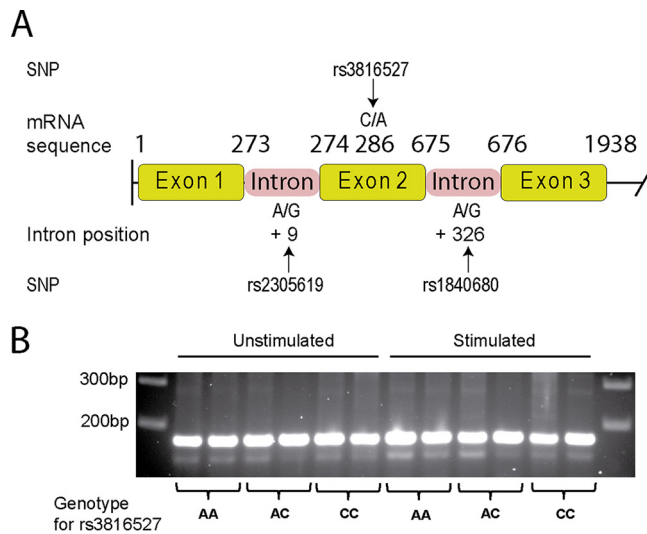
Natural immunity is the first line of defense against infection. However, individual susceptibility to specific microbes is variable and difficult to assess. This makes the identification of host biomarkers to predict the risk of infection by opportunistic microbes an imperative issue. On this basis, the assessment of risk factors associated with polymorphisms in receptors involved in the early recognition of microbes by the innate immune system is of clinical relevance (24).

rs16910526 is the Dectin-1 polymorphism for which loss of function is best explained (14); however, it did not exhibit a correlation with invasive mycosis (*Candida* spp. and *Aspergillus* spp.). Other studies have shown that rs16910526 has a limited influence on susceptibility to invasive aspergillosis, although it might be important in susceptible patients not submitted to HSCT (25, 26). In the present study, a significant protective effect of rs16910526 on fungal colonization was observed in patients receiving AP. Although the relevance of this finding may be tempered by the low frequency of this polymorphism, it is worth noting that G allele carriers displayed a lower rate of fungal colonization (7.5% with fungal colonization versus 18.4% without fungal colonization) and that the rate of colonization was lower in patients with AP than in those who did not receive AP (7.5% and 16.7%, respectively) (see Table S5 in the supplemental material). An interpretation of this finding may be that the benefit obtained from AP can be associated with the prevention of colonization, particularly by *Candida*. However, the redundancy of mechanisms involved in pathogen recognition and the possibility that it may be part of a larger haplotype involving other genetic variants in neighboring genes should be taken into account. In fact, a study using monocytes showed that Dectin-1-defective human macrophages produce proinflammatory cytokines in response to *Aspergillus* (25). This ability to retain the capacity to respond to infection in the absence of functional Dectin-1 highlights the relevance of

**TABLE 10** Possible effects of SNP rs3816527 on alternative splicing of *PTX3* pre-mRNA<sup>a</sup>

3' reference motif	Reference score	Mutated motif	Mutation score	Variation (%)
gtgtgtatcccgtactctagCCA	4.47	gtgtgtatcccgtactctagCCA	4.47	+0
CGCCGTGCG <b>ACT</b> GCGGTcagGAG	1.69	cgccgtgcgctgcggtcagGAG	4.1	<b>+342.6</b>

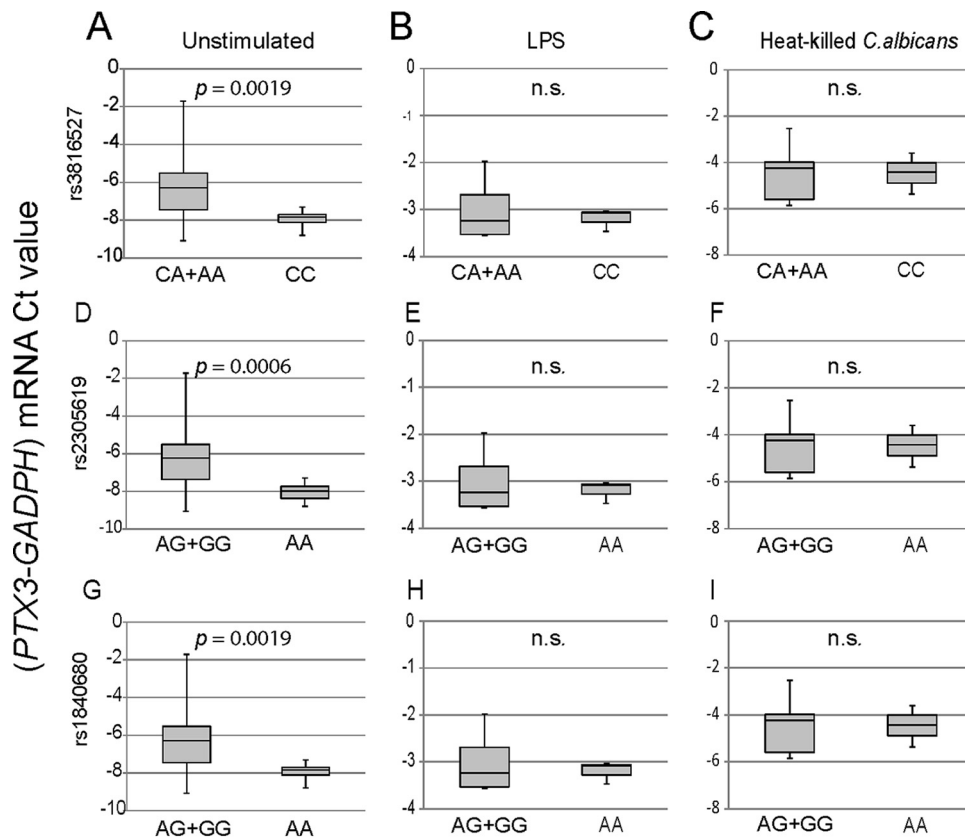
<sup>a</sup>The Human Splicing Finder system allowed the identification and prediction of a new possible cryptic site on *PTX3* pre-mRNA with a high score variation. The C/A change (boldface) generates a cryptic site (italics). As a result, 23 nucleotides of exon 2 (uppercase) become part of the intron sequence (lowercase).



**FIG 3** Structure of the *PTX3* gene, positions of the polymorphisms, and expression of *PTX3* transcripts of the different *PTX3*<sub>rs3816527</sub> genotypes. (A) Structure of the *PTX3* gene and positions of the polymorphisms. The diagram has been constructed based on the sequence reported under GenBank accession number NG\_051000.1. (B) Samples of buffy coats were used for rs3816527 genotyping and processed to obtain mRNA from monocytes cultured in the presence and absence of heat-killed *C. albicans*. *PTX3* transcripts from each sample were generated by RT-PCR and separated by electrophoresis on an agarose gel.

alternative routes and PRRs in antifungal defense (13, 27, 28). Both the low frequency and the presence of alternative mechanisms of defense could also explain the lack of an association of the rs16910631 and rs2078178 Dectin-1 SNPs with fungal infection as well as the surprising correlation between rs2078178 and fungal colonization in patients with AP. These polymorphisms have been associated with ulcerative colitis and Asperger disease. As both pathologies are characterized by the presence of dysbiosis (29, 30), the involvement of these SNPs in pathogenesis would agree with the inability of *Clec7a*<sup>-/-</sup> mice to lead an effective immune response against specific intestinal fungi that might contribute to dysbiosis. However, evidence disclosing whether these polymorphisms influence *CLEC7A* expression has not been reported, and the present study did not show any difference in the expression of *CLEC7A* isoforms that could be associated with these genotypes in human monocytes.

*PTX3* SNPs have been associated with both fungal and bacterial infections, including those caused by *Pseudomonas aeruginosa* and *Mycobacterium tuberculosis* (21, 22), due to the ability of *PTX3* to opsonize microbial surfaces. Regarding fungal infections, invasive mycoses represent a great risk for morbidity and mortality of patients with high-risk hematological malignancies. Although the number of patients with invasive mycosis was relatively low (11.1%), a significant association with *PTX3* polymorphisms was observed, particularly with systemic aspergillosis. Strikingly, this association was maintained in those patients who had received antifungal prophylaxis. Regarding the risk of developing candidiasis, only polymorphism rs1840680 displayed a significant correlation. This highlights the role of *PTX3* in antifungal defense through the opsonization of *Candida* spp. either directly (31) or in combination with mannose-binding lectin (MBL) and complement components (32). In keeping with data from previous reports (10, 11, 21, 22, 33, 34), these results highlight the importance of these polymorphisms in protection against both fungal and bacterial infections, suggesting that *PTX3* expression is a significant risk biomarker. In accordance with our risk alleles, the C allele for rs3816527 and the A allele for both rs2305619 and rs1840680 were associated with pulmonary tuberculosis and *Pseudomonas* species colonization (21, 22). Conversely, risk alleles for invasive mycosis and, particularly, for aspergillosis were the A allele for rs3816527 and the G allele for intronic SNPs, both in solid-organ transplant recipients (33, 34) and in nonneutropenic hematology patients (10, 11). Similar results were



**FIG 4** Impact of PTX3 SNPs on PTX3 mRNA expression. After genotyping and processing of buffy coats, monocytes were cultured in the presence and absence of a stimulus (LPS or heat-killed *C. albicans*) for 6 h. *PTX3* transcripts were amplified by quantitative reverse transcription-PCR in duplicate, and the expression level from each sample was calculated as the difference between the mean  $C_T$  values of the targeted gene (*PTX3*) and the mean  $C_T$  values of the housekeeping gene (*GAPDH*) (y axis, *PTX3*-*GAPDH* mRNA  $C_T$  value). For each SNP, two comparisons of genetic groups were performed: CC and CA+AA for rs3816527 and AA and AG+GG for both intronic SNPs (rs2305619 and rs1840680). The groups passed the Shapiro-Wilks normality test. The groups passed the Shapiro-Wilks normality test. *PTX3* mRNA expression values in monocytes for the indicated genotypes in the absence (A) and presence (B and C) of stimuli are presented in box plot graphs. Results represent data from at least three independent experiments. *P* values determined by Welch's *t* test are shown. n.s., not significant.

observed with risk haplotype A-C-A (rs2305619-rs3816527-rs1840680) in the present study, which agrees with the risk haplotypes obtained in the analyses by Olesen et al. and Chiarini et al. (21, 22) but not with those in other studies of fungal infection (10, 11, 33, 34). Although combined analysis of data from our studies and those from the studies by Cunha et al. (10) and Brunel et al. (11) allowed the establishment of the AA genotype for rs3816527 and the GG genotype for intronic SNPs as risk genotypes, the lack of data did not allow verification of our risk genotype. In addition, despite considering data from the studies by Cunha et al. and Brunel et al. as best fitting with our inclusion criteria, it has to be taken into account that in both studies, the risk association was established in patients without neutropenia, while in the present study, all patients had severe neutropenia, a condition that restricts phagocytic defense to mononuclear cells. These cells exhibit opsonic phagocytosis via CR3 and CR4 integrins and Fc $\gamma$ Rs, but they also express C-type lectin receptors for nonopsonic phagocytosis. In contrast, the unopsonized killing of *Candida* by human polymorphonuclear leukocytes depends not on Dectin-1 but on CR3 (35). The distinct array of receptors involved in fungal recognition in polymorphonuclear leukocytes and mononuclear phagocytes may explain this discrepancy. Thus, as reflected by Egger's *P* value and the trim-and-fill method, further studies of the association between these SNPs and hematology patients, distinguishing those with and those without neutropenia, are needed.

In order to resolve these divergences, the molecular consequences of these SNPs

were assessed. *In silico* studies showed that the exchange of amino acids induced by missense variant rs3816527 did not influence either protein folding or structural stability, in keeping with the report by Cunha et al. showing that the ability to bind fungi was guaranteed with both amino acids (10). However, the RNAfold program showed that mRNA stability could be affected by this exchange of nucleotides, and the Human Splicing Finder tool showed that the presence of the C allele for rs3816527 could create a new cryptic splicing site, which would encompass the loss of 23 bp in exon 2. However, no differences in the sizes of transcripts were found. In contrast, the rs3816527 CC genotype showed lower levels of *PTX3* mRNA expression in resting monocytes than in the CA+AA group. Similarly, in both intronic SNPs, the *PTX3* mRNA expression level was significantly lower in carriers of the AA genotype than in carriers of the AG or GG genotype. These results were consistent with the predicted alterations in the *PTX3* mRNA structure and agreed with the observed clinical associations in our study. In contrast, Cunha et al. (10) observed that the A allele for rs3816527 and the G allele for rs2305619 showed lower levels of *PTX3* mRNA in neutrophil precursors. In keeping with our data, these samples belonged to healthy individuals who showed no infections. To relate the mRNA expression data with the response to pathogen-associated molecular patterns (PAMPs), monocytes were cultured in the presence of both heat-killed *C. albicans* and LPS. It could be posited that the difference between genotypes would remain after stimulation, according to the different rates of infection incidence. However, no significant differences in the extents of *PTX3* mRNA induction were found. An overall interpretation could be that a low expression level of *PTX3* in mononuclear phagocytes at the time of initial contact with the fungal burden may be critical to cope successfully with the cargo and to control the spread of invasion, given the limited supply of polymorphonuclear leukocytes in these patients. It is also possible that *PTX3* deficiency adversely affects antifungal mechanisms mediated by other innate immune receptors (36) or the mobilization of phagocytes (37). Given the various PAMPs expressed in fungi and the formation of a phagocytic synapse in mononuclear phagocytes, critical for fungal recognition (38), it seems likely that the presence of *PTX3* in the initial phase of infection might be a crucial factor for a proper response. Even though no modifications in the *PTX3* structure were recognized as a result of the D48A substitution in rs3816527, damage to its electrostatic potential or interactions with other proteins cannot be ruled out. The fact that the 48th position is fringed by two cysteine residues involved in the formation of *PTX3* protein complex octamers supports this possibility. To establish a better correlation between *PTX3* RNA expression and infection, patient samples should have been collected during an infection period. However, the clinical protocol was designed to use a small amount of peripheral blood at the time of high-risk hematological malignancy diagnosis. Despite this limitation in this retrospective study, the clinical results confirm the significant role of *PTX3* in defense against fungi, particularly against *Aspergillus* spp., and highlight the presence, in a homozygous state, of the C allele for rs3816527 and of the A allele for rs2305619 and rs1840680 as hazard factors in high-risk hematology patients (both transplanted and nontransplanted). However, given the discrepancies with some of the previously reported results, further investigations are warranted to establish the real risk genotype in *PTX3* SNPs under definite criteria for patient enrollment. This would facilitate early prophylaxis and appropriate treatment in patients with high-risk hematological malignancies. A corollary to this study is that genetic associations with a particular disease should be established with caution, given the difficulty in replicating data from clinical studies due to the involvement of populations that differ in terms of baseline characteristics and/or immunosuppressive regimens (5).

## MATERIALS AND METHODS

**Study populations.** This is a retrospective study of a group of 198 patients with high-risk hematological malignancies enrolled between 2013 and 2017 from both the Hospital Universitario Río-Hortega (HURH) and the Hospital Clínico Universitario of Valladolid (HCUV). Clinical diagnoses included acute leukemias, high-risk myelodysplastic syndromes, highly aggressive lymphomas, and chronic lymphoblastic leukemia. Patients who underwent HSCT were also included in the study. The inclusion criteria were

intensive chemotherapy and/or high doses of corticoids and episodes of severe neutropenia ( $<500$  cells/mm<sup>3</sup>) for at least 7 days. The observational period was defined as the interval between the date of the initial high-risk diagnosis and the end of clinical monitoring. Bacterial, viral, and fungal infections were monitored, as were the prophylactic and therapeutic strategies employed. Fungal infections were detected mainly by microscopy and culture and diagnosed based on updated criteria reported by the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group (EORTC/IFICG) (39). Clinical and laboratory data were obtained through detailed review of hospital records.

For genetic studies, the time of extraction of the samples was decided by clinical criteria at the time of establishment of a high-risk diagnosis. For transplant patients, only samples collected prior to HSCT were studied. For complementary functional studies, buffy coat samples from healthy volunteer donors were provided by the Centro de Hemoterapia y Hemodonación de Castilla y León. All determinations and genetic analyses in hematological patients as well as the use of buffy coat samples were performed after obtaining written informed consent in accordance with the Declaration of Helsinki, and anonymity of the data was guaranteed. The study protocol for patients was approved by the ethical review committees of both hospitals.

**Genotyping.** Genomic DNA was extracted from 400- $\mu$ l samples of peripheral blood or buffy coats from healthy donors, using MagNA Pure compact nucleic acid isolation kit I (Roche, Mannheim, Germany). Genotyping of *CLEC7A* and *PTX3* polymorphisms studied here was performed by using the Competitive allele-specific PCR (KASP) assay. Primers, designed by using Primer-BLAST software, are shown in Table S8 in the supplemental material. Samples of 4 ng genomic DNA were amplified by using a Roche LightCycler 480 instrument (Roche Austria, Vienna, Austria). PCR fluorescent endpoint readings were performed by using the LightCycler 480 real-time PCR system (Roche, Germany).

**Isolation of human monocytes.** Human monocytes were isolated from buffy coats of healthy donors by two different density gradient centrifugations: a first centrifugation using a Ficoll-Paque solution (GE Healthcare Bio-Sciences, Uppsala, Sweden) to obtain mononuclear cells and a second centrifugation based on a discontinuous density gradient constituted by an OptiPrep solution (Axis-Shield PoC AC, Oslo, Sweden), a Ficoll-Paque solution, and a mixture composed of HEPES-buffered saline containing OptiPrep, bovine serum albumin (BSA), and 1 mM EDTA, in order to separate lymphocytes from monocytes. Monocytes were collected and adhered to 100-mm plastic dishes for 2 h at 37°C to discard nonadherent cells and guarantee a high purity of monocytes. Monocytes were cultured overnight at a density of  $1 \times 10^7$  cells/plate in RPMI 1640 medium (Lonza, Vervier, Belgium) supplemented with 2 mM L-glutamine, 100 U/ml penicillin-streptomycin, and 5% heat-inactivated human serum. To address the changes in the expression of *PTX3* upon stimulation by pathogen-associated molecular patterns (PAMPs), adhered monocytes were stimulated for 6 h with 10  $\mu$ g/ml lipopolysaccharide (LPS) and heat-killed *Candida albicans* at a concentration of 5 conidia per cell. At the end of this period, RNA was isolated and used for the assay of *PTX3* mRNA expression by reverse transcription and real-time PCR (RT-PCR).

**Pathogenicity prediction for *PTX3* SNPs.** The influence of the D48A substitution associated with the exonic SNP rs3816527 on *PTX3* folding and structural stability was studied with the SIFT program (<http://sift.jcvi.org/>). The secondary structure of single-stranded RNA was predicted by using RNAfold (<http://rna.tbi.univie.ac.at/cgi-bin/RNAWebSuite/RNAfold.cgi>), while the potential splice-site effects of SNPs were predicted by using the Human Splicing Finder tool (<http://www.umd.be/HSF3/HSF.shtml>).

**Analysis of mRNA expression.** Total RNA from healthy control monocytes was obtained by using TRIzol-chloroform extraction according to the manufacturer's instructions and then used for reverse transcription. RT-PCR was performed in duplicate by using a Kapa SYBR fast quantitative PCR (qPCR) kit optimized for the LightCycler 480 system (Kapa Biosystems, Boston, MA, USA) on a Roche LightCycler 480 instrument. Amplification efficiencies were validated and normalized against the value for the glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) gene. Relative expression levels of both *PTX3* and *CLEC7A* mRNAs were calculated as the difference between the mean threshold cycle ( $C_t$ ) values of targeted genes and the mean  $C_t$  values of the housekeeping gene (*GAPDH*). The primer sequences, designed by using Primer3 software (<http://bioinfo.ut.ee/primer3-0.4.0/>), are listed in Table S9 in the supplemental material. In addition, PCR products were separated by electrophoresis on agarose gels stained with gel red nucleic acid gel stain.

**Statistical analysis.** Descriptive analysis of patients was performed by using IBM SPSS Statistics 20 (IBM, Chicago, IL, USA). The clinical variables were assessed, and their absolute values, frequencies, and percentages are presented. The microorganisms so far associated with the response orchestrated by Dectin-1 or *PTX3* were considered appropriate groups to assess the possible influence of SNPs. These microorganisms included cytomegalovirus, the genus *Pseudomonas*, the genus *Staphylococcus*, *Klebsiella pneumoniae*, *Mycobacterium tuberculosis*, the genus *Candida*, and the genus *Aspergillus*. Genotypic analysis of the different SNPs was carried out to assess HWE, allele and genotype distributions and association tests. This was performed by using the SNPStats program (<http://bioinfo.iconcologia.net/SNPStats>). This Web application establishes the most frequent genotype as the default reference and generates odds ratios (OR), 95% confidence intervals (CI), and *P* values for the three main inheritance models: codominant, dominant, and recessive. Model fit was evaluated based on the Bayesian information criterion (BIC), with a lower BIC value indicating a better fit. All analyses were adjusted for sex, age, hematological malignancy, and antifungal prophylaxis in order to discard these possible confusion covariates. Finally, haplotype frequencies of *PTX3* SNPs and their association risk with different infection groups were also tested by using this Web tool.



**Systematic review with meta-analysis for PTX3 SNPs.** A literature search of published studies in the PubMed database was performed by using the keywords “PTX3,” “polymorphisms,” and “infections.” Of the 11 studies obtained, only 2 reports were selected under the following inclusion criteria: (i) study SNPs (rs2305619, rs3816527, and rs1840680), (ii) type of study (analysis of the association of *PTX3* SNPs with fungal infection), and (iii) patients (patients with hematological disease). A flow chart showing the stages of database searching and study selection is provided in Fig. S3 in the supplemental material. The OR from different studies were used to assess the influence of the reported SNPs on invasive aspergillosis in hematology patients. The heterogeneity of studies was checked by using Cochran’s chi-square test (Q-test), considering a *P* value of <0.05 to be statistically significant heterogeneity, and the *I*<sup>2</sup> statistic, where an *I*<sup>2</sup> value of >75% was considered to indicate high heterogeneity. A random-effects-model meta-analysis was performed for each SNP, as recommended previously (40). We used the Der Simonian-Laird approach to estimate the variance between studies. Finally, publication bias was assessed by using a funnel plot, with filled studies estimated from the trim-and-fill method, and the Egger regression asymmetry test. All meta-analyses were conducted with the metafor R package, version 2.0-0.

## SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/IAI.00709-18>.

**SUPPLEMENTAL FILE 1**, PDF file, 0.2 MB.

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We declare no conflict of interest.

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