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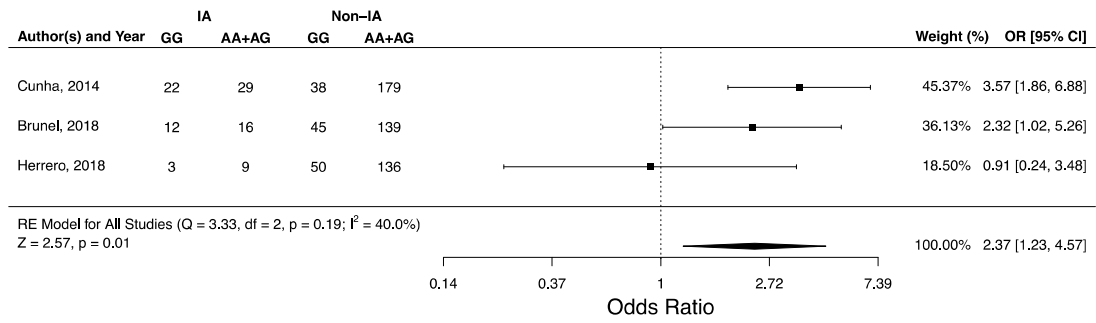 Polymorphisms in Receptors Involved in Opsonic and Nonopsonic Phagocytosis, and the Risk of Infection in Oncohematology Patients

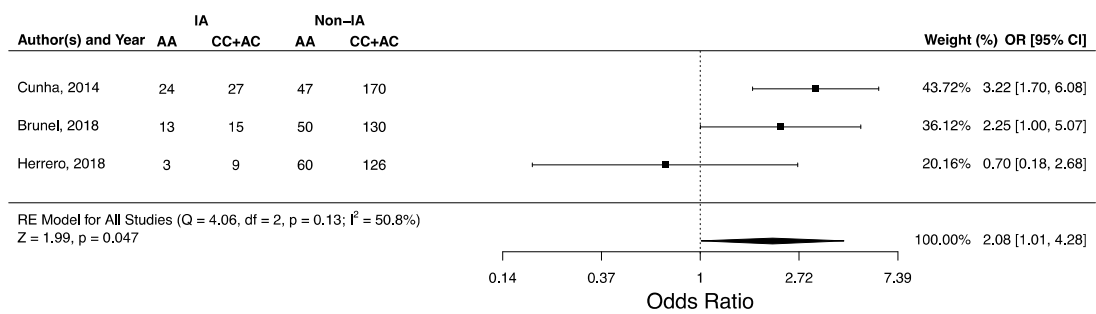Mª Carmen Herrero-Sánchez, Eduardo B. Angomás, Cristina de Ramón, Juan J. Tellería, Luis A. Corchete, Sara Alonso, Mª del Carmen Ramos, María J. Peñarrubia, Saioa Márquez, Nieves Fernández, Luis J. García Frade, Mariano Sánchez Crespo

Supplementary data

A

Author(s) and Year	IA		Non–IA		Weight (%)	OR [95% CI]
	GG	AA+AG	GG	AA+AG		
Cunha, 2014	22	29	38	179	45.37%	3.57 [1.86, 6.88]
Brunel, 2018	12	16	45	139	36.13%	2.32 [1.02, 5.26]
Herrero, 2018	3	9	50	136	18.50%	0.91 [0.24, 3.48]

RE Model for All Studies (Q = 3.33, df = 2, p = 0.19; I^2 = 40.0%)
Z = 2.57, p = 0.01 — 100.00% 2.37 [1.23, 4.57]

0.14   0.37   1   2.72   7.39
Odds Ratio

B

Author(s) and Year	IA		Non–IA		Weight (%)	OR [95% CI]
	AA	CC+AC	AA	CC+AC		
Cunha, 2014	24	27	47	170	43.72%	3.22 [1.70, 6.08]
Brunel, 2018	13	15	50	130	36.12%	2.25 [1.00, 5.07]
Herrero, 2018	3	9	60	126	20.16%	0.70 [0.18, 2.68]

RE Model for All Studies (Q = 4.06, df = 2, p = 0.13; I^2 = 50.8%)
Z = 1.99, p = 0.047 — 100.00% 2.08 [1.01, 4.28]

0.14   0.37   1   2.72   7.39
Odds Ratio

C

Author(s) and Year	IA		Non–IA		Weight (%)	OR [95% CI]
	GG	AA+AG	GG	AA+AG		
Cunha, 2014	22	29	38	179	44.20%	3.57 [1.86, 6.88]
Brunel, 2018	12	16	45	139	36.19%	2.32 [1.02, 5.26]
Herrero, 2018	3	9	53	133	19.61%	0.84 [0.22, 3.21]

RE Model for All Studies (Q = 3.71, df = 2, p = 0.16; I^2 = 46.1%)
Z = 2.34, p = 0.019 — 100.00% 2.30 [1.14, 4.61]
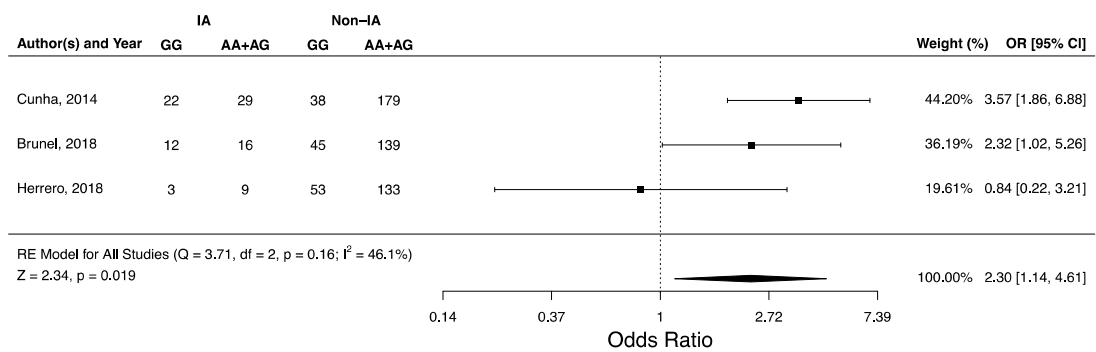
0.14   0.37   1   2.72   7.39
Odds Ratio

FIG S1 Forest plots of association between *PTX3* SNPs and invasive aspergillosis (IA) in hematological patients. For each *PTX3* SNPs, a forest plot showing the odds ratio of each study using a random effects model meta-analysis. Summary estimated odds ratio are represented as a diamond at the bottom for each analysed SNP. (A) rs2305619, (B) rs3816527 and (C) rs1840680.
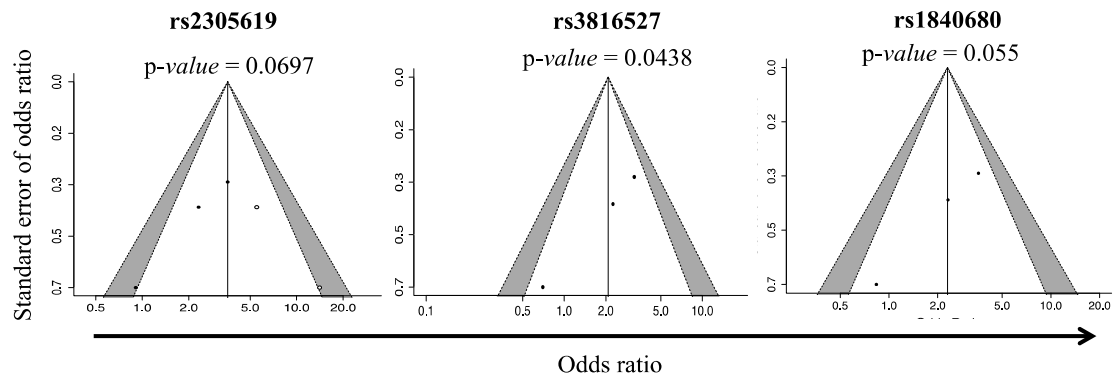
FIG S2 Funnel plots of association between *PTX3* SNPs and IA. For each *PTX3* SNPs, a funnel plot to identify publication bias was produced. Egger's asymmetry test p-value was also shown for each funnel plot.
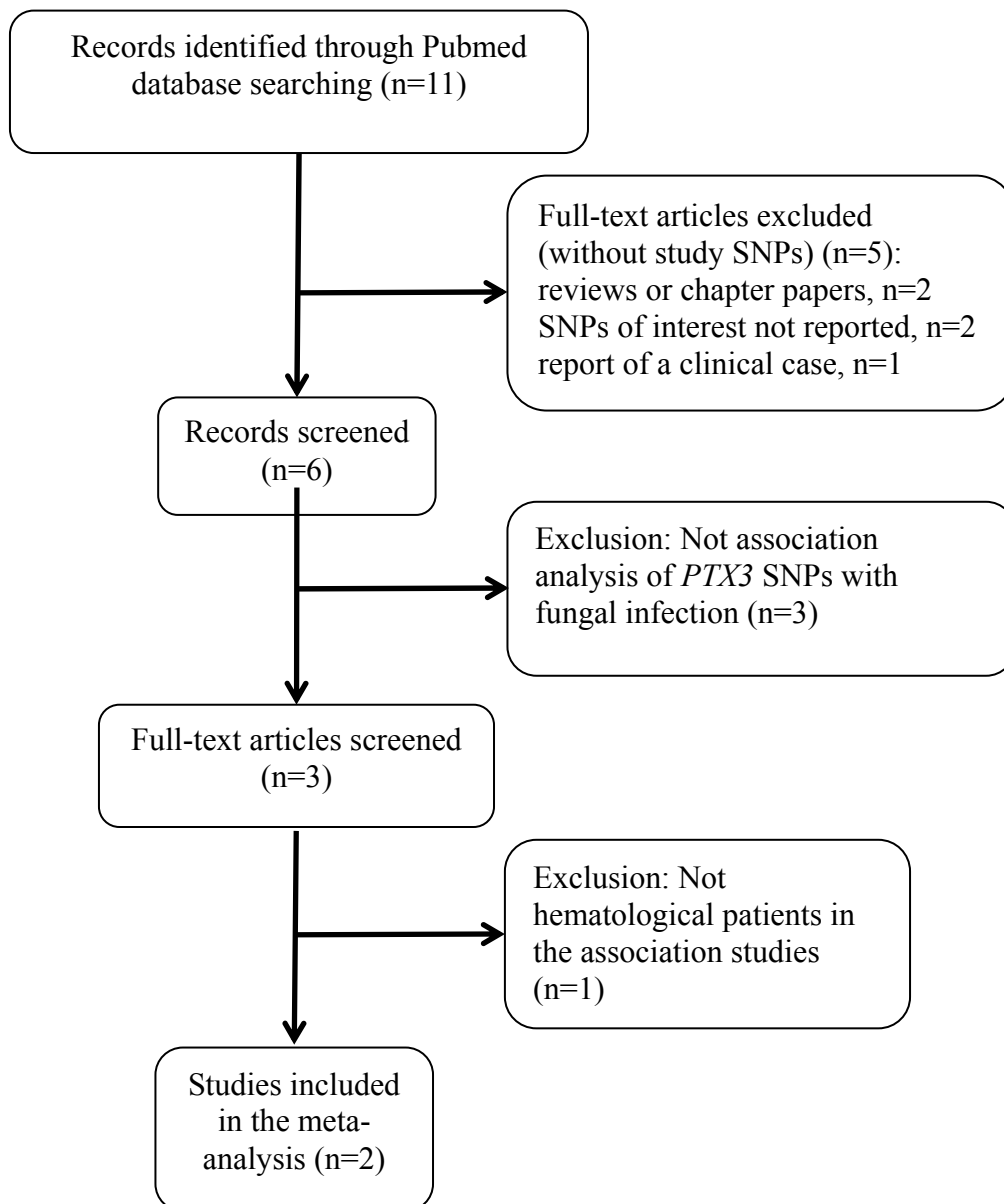
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┌─────────────────────────────────┐
│ Records identified through Pubmed│
│ database searching (n=11)        │
└─────────────────────────────────┘
                │
                │         ┌──────────────────────────────────┐
                │         │ Full-text articles excluded       │
                │         │ (without study SNPs) (n=5):       │
                ├────────▶│ reviews or chapter papers, n=2    │
                │         │ SNPs of interest not reported, n=2│
                │         │ report of a clinical case, n=1    │
                ▼         └──────────────────────────────────┘
       ┌──────────────────┐
       │ Records screened  │
       │ (n=6)             │
       └──────────────────┘
                │
                │         ┌──────────────────────────────────┐
                │         │ Exclusion: Not association         │
                ├────────▶│ analysis of PTX3 SNPs with         │
                │         │ fungal infection (n=3)             │
                ▼         └──────────────────────────────────┘
       ┌──────────────────────┐
       │ Full-text articles     │
       │ screened               │
       │ (n=3)                  │
       └──────────────────────┘
                │
                │         ┌──────────────────────────────────┐
                │         │ Exclusion: Not                     │
                ├────────▶│ hematological patients in          │
                │         │ the association studies            │
                │         │ (n=1)                              │
                ▼         └──────────────────────────────────┘
       ┌──────────────────┐
       │ Studies included  │
       │ in the meta-      │
       │ analysis (n=2)    │
       └──────────────────┘
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FIG S3 Flow chart of study selection in meta-analysis

TABLE S1 Genotype distribution of rs16910526 in patients presenting *Candida spp* and *Aspergillus* spp.

rs_number	Model	Genotype	With CAD (%)	Without CAD (%)	OR (95% CI)	P-value	BIC
rs16910526	Codominant	TT	80 (89.9%)	89 (81.7%)	1.00		
		TG	8 (9%)	18 (16.5%)	2.38 (0.94-6.02)	0.16	318.4
		GG	1 (1.1%)	2 (1.8%)	1.6 (0.13-20.16)		
	Dominant	TT	80 (89.9%)	89 (81.7%)	1.00		
		TG+GG	9 (10.1%)	20 (18.4%)	2.28 (0.95-5.51)	0.058	313.2
	Recessive	TT+TG	88 (98.9%)	107 (98.2%)	1.00		
		GG	1 (1.1%)	2 (1.8%)	1.45 (0.12-18.3)	0.77	316.7

Association analysis for the 198 patients was adjusted for sex, age, and hematological malignancy. Abbreviations: **CAD**, *Candida* spp and/or *Aspergillus* spp detection; **OR**, odds ratio; **CI**, confidence intervals; **BIC**, Bayesian information criterion.

TABLE S2 Association of SNP rs16910526 with fungal colonization

rs_number	Model	Genotype	With FC (%)	Without FC (%)	OR (95% CI)	P-value	BIC
rs16910526	Codominant	TT	64 (90.1%)	89 (82.7%)	1.00		
		TG	6 (8.4%)	20 (15.8%)	2.61 (0.93-7.32)	0.15	299.4
		GG	1 (1.4%)	2 (1.6%)	1.12 (0.1-14.48)		
	Dominant	TT	64 (90.1%)	105 (82.7%)	1.00		
		TG+GG	7 (9.9%)	22 (17.3%)	2.37 (0.9-6.21)	0.066	294.5
	Recessive	TT+TG	70 (98.6%)	125 (98.4%)	1.00		
		GG	1 (1.4%)	2 (1.6%)	1.02 (0.1-13.14)	0.99	297.9

Model adjusted for sex, age, and hematological malignancy for high-risk hematological patients with and without fungal colonization by *Aspergillus* spp. and *Candida* spp. Abbreviations: **FC**, fungal colonization.

TABLE S3 Fungal colonization and SNP rs16910526 in patients receiving antifungal prophylaxis

rs_number	Model	Genotype	With FC (%)	Without FC (%)	OR (95% CI)	P-value	BIC
rs16910526	Codominant	TT	49 (92.5%)	71 (81.6%)	1.00		
		TG	4 (7.5%)	14 (16.1%)	3.7 (1.01-13.54)	0.028	210.8
		GG	0 (0%)	2 (2.3%)	NA (0.00-NA)		
	Dominant	**TT**	**49 (92.5%)**	**71 (81.6%)**	**1.00**		
		TG+GG	**4 (7.5%)**	**16 (18.4%)**	**4.36 (1.22-15.6)**	**0.013**	**206.8**
	Recessive	TT+TG	53 (100%)	85 (97.7%)	1.00		
		GG	0 (0%)	2 (2.3%)	NA (0.00-NA)	0.1	210.4

Association analysis for the 140 patients undergoing antifungal prophylaxis was adjusted for sex, age, and hematological malignancy, using SNPStats program.

TABLE S4 Genotype association of rs16910526 in hematological patients receiving antifungal prophylaxis and undergoing colonization by *Candida* spp.

rs_number	Model	Genotype	With CC (%)	Without CC (%)	OR (95% CI)	P-value	BIC
rs16910526	Codominant	TT	43 (91.5%)	77 (82.8%)	1.00		
		TG	4 (8.5%)	14 (15.1%)	3.04 (0.8-11.44)	0.076	205.3
		GG	0 (0%)	2 (2.1%)	NA (0.00-NA)		
	Dominant	TT	43 (91.5%)	77 (82.8%)	1.00		
		TG+GG	4 (8.5%)	16 (17.2%)	3.54 (0.97-12.9)	0.038	201.2
	Recessive	TT+TG	47 (100%)	91 (97.8%)	1.00		
		GG	0 (0%)	2 (2.1%)	NA (0.00-NA)	0.15	203.4

Genetic association study adjusted for sex, age, and hematological malignancy, using SNPStats program. Abbreviations: **CC**, *Candida* colonization.

TABLE S5 Genotype frequency of SNP rs16910526 in patients with and without antifungal prophylaxis

Genotype	140 patients AP		58 patients NO AP	
	With colonization	Without colonization	With colonization	Without colonization
TT	49 (92.5%)	71 (81.6%)	15 (83.3%)	34 (84%)
TG	4 (7.5%)	14 (16.1%)	2 (11.1%)	5 (15%)
GG	0 (0%)	2 (2.3%)	1 (5.6%)	0 (0%)

Genotype distribution and the incidence of fungal colonization were assessed in patients with and without AP.

TABLE S6 Genotype association between SNP rs2078178 and fungal colonization in patients with antifungal prophylaxis

rs_number	Model	Genotype	With FC (%)	Without FC (%)	OR (95% CI)	P-value	BIC
rs16910526	Codominant	CC	37 (69.8%)	44 (50.6%)	1.00		
		TC	15 (28.3%)	39 (44.8%)	2.02 (0.92-4.47)	0.062	212.4
		TT	1 (1.9%)	4 (4.6%)	6.85 (0.64-72.9)		
	Dominant	CC	37 (69.8%)	44 (50.6%)	1.00		
		TC+TT	16 (30.2%)	43 (49.4%)	2.25 (1.04-4.87)	0.036	208.7
	Recessive	CC+TC	52 (98.1%)	83 (95.4%)	1.00		
		TT	1 (1.9%)	4 (4.6%)	5.33 (0.5-55.06)	0.12	210.6

Association studies for SNP rs2078178 in the 140 patients undergoing antifungal prophylaxis. Analysis adjusted for sex, age, and hematological malignancy.

TABLE S7 Tolerance analysis for the substitution of an aspartic acid for an alanine in the 48 position as a consequence of polymorphism rs3816527

In silico study was performed by SIFT program using orthologues in the protein alignment (**A**) and homologues in the protein alignment (**B**)

A

SNP	Amino acid change	Protein ID	Amino acid	Using orthologues in the protein alignment			
				Prediction	Score	Median Info	Alignment
rs3816527	A48D	NP_002843	A	TOLERATED	1.00	3.18	Alignment of best hits
			D	TOLERATED	0.59		

B

SNP	Amino acid change	Protein ID	Amino acid	Using orthologues in the protein alignment			
				Prediction	Score	Median Info	Alignment
rs3816527	A48D	NP_002843	A	TOLERATED	1.00	3.18	Alignment of best hits
			D	TOLERATED	0.59		

TABLE S8 Primer sequences used for KASP assays

Gene	rs_number	Primer sequences
CLEC7A	rs16910631	Labeled 5`-TCAAAGGATTATTGCGGGAATTAAA[T/C-]3´[HEX/FAM] Common 5`-AAGGTGCTTTTATATGTGTTAGCTC-3´
	rs2078178	Labeled 5´-CCATGAAAACTGCCTAGGGGGACTG[C/T]-3´[HEX/FAM]) Common 5`-AACCTATCTGACAAAATCCCAGATG-3´
	rs16910526	Labeled 5`-ACCAACTGTGTAGTGTGCCCTCATA[G/T]-3´[HEX/FAM] Common 5`-TTGAAAACTTCTTCTCACAAATACT-3´
PTX3	rs2305619	Labeled 5`-CCATCCCACTGAGGACCGTAAGTTC[G/A]-3´[HEX/FAM] Common 5`-AGGGTTAGCAGAGAAACAGTTAAAG-3´
	rs3816527	Labeled 5´-TCCCGTACTCTAGCCACGCCGTGCG[C/A]-3´[HEX/FAM] Common 5`-CCATTCCGAGTGCTCCTGACCGCAG-3´
	rs1840680	Labeled 5´-GGGCCCCTTACACCCGATCCGACTA[G/A]-3´[HEX/FAM]) Common 5`-ACGGGCCAGTGGACTCCTGCAGCGC-3´

TABLE S9 Primer sequences used for amplification of *CLEC7A*, *PTX3* and *GAPDH*

genes

Gene	Primer	Sequence
CLEC7A	Forward	5´-GGGCTCTCAAGAACAATGGA-3'
	Reverse	5´-TGAGGGCACACTACACAGTTG-3'
PTX3	Forward	5´-TGCGATTCTGTTTTGTGCTC-3'
	Reverse	5´-TGAAGAGCTTGTCCCATTCC-3'
GAPDH	Forward	5´-GTCAGTGGTGGACCTGACCT-3'
	Reverse	5´-AGGGGAGATTCAGTGTGGTG-3'