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Mini Review

Association between polymorphisms in the genes encoding toll-like receptors and dectin-1 and susceptibility to invasive aspergillosis: a systematic review

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Abstract

Invasive aspergillosis is a common fungal infection in immunocompromised individuals. Some studies have shown that toll-like receptor and dectin-1 genetic polymorphisms may alter signaling pathways, thus increasing an individual's susceptibility to invasive aspergillosis. We investigated the pertinent literature to determine whether polymorphisms in the genes encoding toll-like receptors and dectin-1 increase the susceptibility to invasive aspergillosis. This study systematically reviewed the literature using the databases PubMed/PMC, Scopus, and Web of Science using the keywords invasive aspergillosis, polymorphism, Toll-like, and Dectin-1. From the initial search, 415 studies were found and according to our inclusion and exclusion criteria, eight studies were selected. Several studies described single-nucleotide polymorphisms (SNPs) that are associated with a greater susceptibility to invasive aspergillosis. These SNPs were found in the genes that encode toll-like receptors 1, 3, 4, and 5 and the gene that encodes dectin-1; upon activation, both cellular receptors initiate a signaling cascade that can result in the production of cytokines and chemokines. Thus, our literature review uncovered a significant association between polymorphisms in the genes that encode toll-like receptors and dectin-1 and invasive aspergillosis. More studies should be performed to better understand the relationship between toll-like receptor and dectin-1 genetic polymorphisms and invasive aspergillosis susceptibility.

Keywords: Invasive aspergillosis. Genetic polymorphism. Toll-like. Dectin-1. Susceptibility.

Invasive infections caused by *Aspergillus* spp. are associated with high rates of morbidity and mortality. The disease develops mainly in patients with hematological malignancies or neutropenia, those treated with corticosteroids or immunosuppressive drugs, or who underwent bone marrow or solid organ transplants[1,2]. Among the factors described above, the most important risk factor for the development of invasive aspergillosis (IA) is neutropenia[3,4]. However, because some patients with similar immunodepression levels develop the disease and others do not, such an association is unclear[1].

Epidemiological studies indicate that a combination of several factors help to determine the probability of developing IA, and one of these factors is genetic. Despite the different genomic variations that may occur, pathologies are usually associated with gene polymorphisms[5,6]. The initial recognition of different pathogens occurs through cellular receptors, called pattern recognition receptors (PRRs), present in cells associated with innate immunity. These receptors recognize pathogen-associated molecular patterns (PAMPs) in the fungal cell wall, subsequently initiating a signaling cascade that can result in the production of cytokines and chemokines. Cytokines and chemokines stimulate the recruitment of neutrophils and consequently antigen-specific immunity occurs[7,8].

Several studies have described the association between IA and polymorphisms of the genes that encode toll-like receptors[9] and dectin-1 (*CLEC7A*)[11]. Such polymorphisms may alter signaling pathways, which increases an individual's susceptibility to developing the disease[10]. Thus, this study aims to evaluate whether individuals with polymorphisms in genes that encode toll-like receptors and dectin-1 are susceptible to IA.

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LITERATURE AND SEARCH STRATEGY

This descriptive bibliographical review obtained its data through an active search of research databases (PubMed/PMC, Scopus, and Web of Science). The data were analyzed using the preferred reporting items for systematic reviews and meta-analyses (PRISMA) recommendations and also included an analysis of the textual description.

The literature search and data collection were performed using a search protocol with the following criteria: subject interest, inclusion criteria, search strategy and data selection, analysis and results presentation and interpretation. The following keywords were used to search in PubMed from the National Center for Biotechnology Information, USA (NCBI): (((Aspergillosis) AND (polymorphism)) AND (Dectin-1) AND/OR (Toll-like))). This same combination of words was used to search in the Scopus and Web of Science databases. Articles written in English and published from January 2008 to December 2017 were included in the present review. The PubMed/PMC, Scopus, and Web of Science databases were chosen because they are comprehensive and internationally used in the health sciences.

References from revision papers and consensuses were manually collected to ensure the inclusion of all relevant papers. No contact was made with clinical investigators to verify possible research in progress.

STUDY SELECTION, DATA EXTRACTION, AND QUALITY OF EVIDENCE

The literature search process, from localization to paper selection, was independently performed by three of the authors in this study. Relevant data were extracted, and differences were resolved by consensus. Potentially eligible articles were obtained and read in entirety. A fourth author was used to decide about the inclusion of a particular article if doubt existed.

The quality of the studies was assessed using the grading of recommendations, assessment, development, and evaluations (GRADE)[9]. The quality of the evidence presented in the studies was classified into four categories: high, moderate, low, or very low quality[10]. We also analyzed the influence of possible conflicts of interest and information on the ethical approval of the studies[11].

The literature search was executed regarding the main themes: the association of polymorphisms in genes coding for the dectin-1 receptor and the toll-like receptors with susceptibility to IA.

Initially, 415 articles were found in the searches; 38 of these studies were selected based on their titles and abstract content. The criteria used for the final selection included: articles closely related to the theme and articles published in the last 10 years. Based on these criteria, eight articles were finally selected (**Figure 1**). The selection information about the studies used is described in **Table 1**.

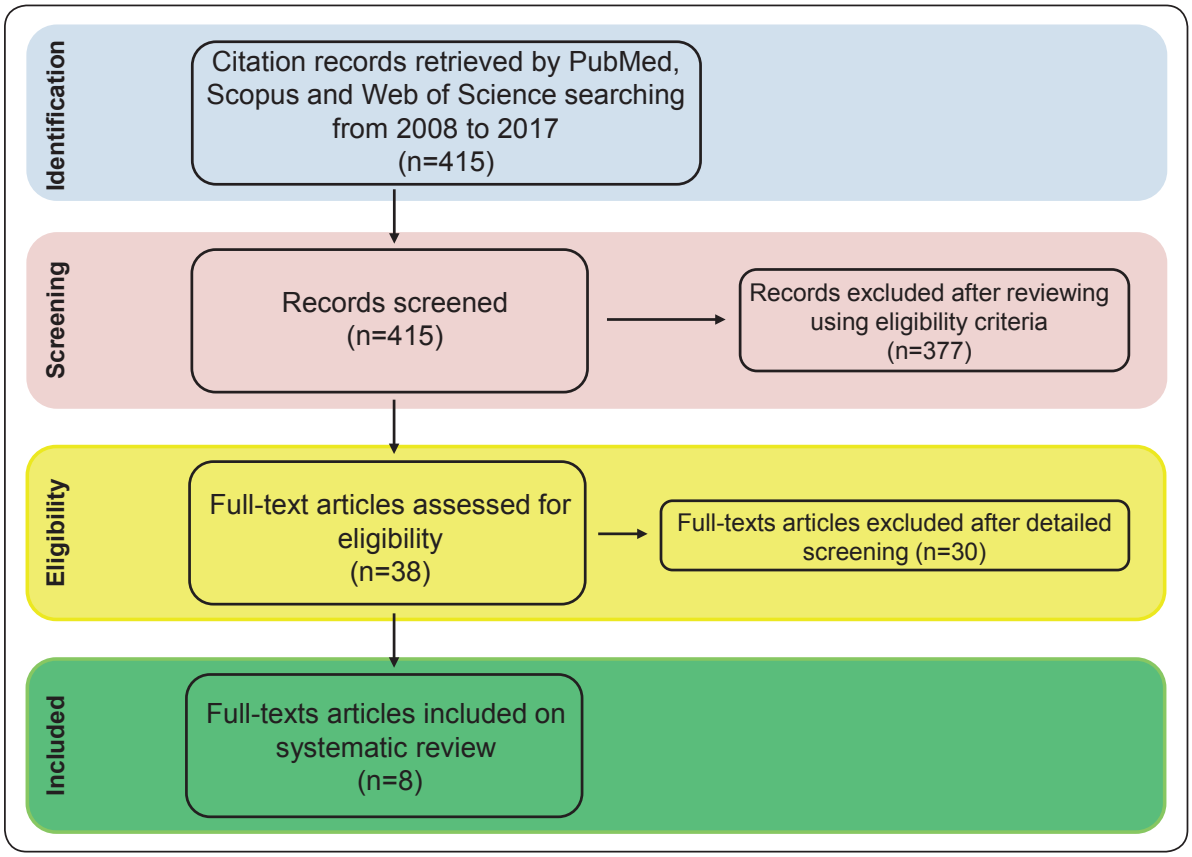

FIGURE 1: Flow diagram of the systematic review of articles published in the last 10 years about the association of polymorphisms in genes encoding toll-like receptors and dectin-1 with susceptibility to aspergillosis.

TABLE 1: Data of selected articles investigating the association of polymorphisms in genes encoding the toll-like receptors and dectin-1 with susceptibility to aspergillosis.

Authors	Year	Title	Type of Study	Study Location
Carvalho et al.[14]	2008	*Polymorphisms in Toll-Like Receptor Genes and Susceptibility to pulmonary Aspergillosis*	Cohort study	United Kingdom
Cunha et al.[1]	2010	*Dectin-1 Y238X polymorphism associates with susceptibility to invasive aspergillosis in hematopoietic transplantation through impairment of both recipient and donor dependent mechanisms of antifungal immunity*	Control case	Italy
Boer et al.[19]	2011	*Influence of Polymorphisms in Innate Immunity Genes on Susceptibility to Invasive Aspergillosis after Stem Cell Transplantation*	Cohort study	Netherlands
Chai et al.[18]	2011	*The Y238X Stop Codon Polymorphism in the Human b-Glucan Receptor Dectin-1 and Susceptibility to Invasive Aspergillosis*	Control case	Belgium
Carvalho et al.[20]	2012	*TLR3 essentially promotes protective class I–restricted memory CD8_ T-cell responses to Aspergillus fumigatus in hematopoietic transplanted patients*	Retrospective cohort study	Italy
Sainz et al.[11]	2012	*Dectin-1 and DC-SIGN Polymorphisms Associated with Invasive Pulmonary Aspergillosis Infection*	Prospective cohort study	England
Grube et al.[13]	2013	*TLR5 stop codon polymorphism is associated with invasive aspergillosis after allogeneic stem cell transplantation*	Control case	Germany
Smith et al.[21]	2014	*Reduced expression of TLR3, TLR10 and TREM1 by human macrophages in Chronic cavitary pulmonary aspergillosis, and novel associations of VEGFA, DENND1B and PLAT*	Control case	Italy

Because it affects patients with hematological diseases and who are immunocompromised, IA has been the target of several immunogenetic studies. Thus, several studies have been carried out to identify the role of genetic polymorphisms in the pathology of IA[12-15]. The main results of the eight articles included in this systematic review are presented in **Tables 2** and **3**.

According to Grube et al.[16], *TLR* genes have been associated with an increased susceptibility to IA primarily in patients who underwent autologous bone marrow transplantation. However, a study that evaluated 127 patients who underwent allogeneic stem cell transplantation (22 = IA, 105 = control cases) found a significant association between the *TLR1* 239 C/G (rs5743611) and 743 A/G (rs4833095) genetic polymorphisms with invasive aspergillosis (OR = 1.30, 95% CI = 1.13–1.50, $p < 0.001$).

In the study performed by Grube et al.[16], single nucleotide polymorphisms (SNPs) of both the recipients and allogeneic bone marrow transplant donors were evaluated, and no statistical relevance was observed for the association between *TLR2*, *TLR4*, *TLR9* and *TLR5* and IA in the donor patients.

Carvalho et al.[17] also developed a study that sought to evaluate the association of IA with *TLR2*, *TLR4*, and *TLR9*. In agreement with the study by Grube et al.[16], Carvalho et al.[17] did not find statistically relevant data associating the *TLR2* gene polymorphism with the disease. However, due to the limited number of patients used in the study, the authors admitted that the importance of the *TLR2* SNP in patients with IA should not be ruled out.

On the other hand, a study that evaluated 120 individuals (40 = IA, 80 = control cases) found that the *TLR4* genetic polymorphism rs4986790 was associated with increased susceptibility to IA pathology (OR = 3.5, 95% CI = 1.5–8.1, $p = 0.003$)[17]. This is justified because the TLR4 receptor is among the main receptors involved in the recognition of pathogenic fungi leading to the activation of the inflammatory response[18,19]. However, according to Pamer[20], this finding is surprising because the *TLR4* receptor is mainly related to the recognition of bacterial lipopolysaccharides. An explanation for such an association would be that the TLR4 receptor can also recognize other molecules, such as the beta glucan present in the cell wall of the fungus, since *Aspergillus* spp. do not produce lipopolysaccharides.

An increased risk of IA development has also been associated with polymorphisms in *TLR5* (rs5744168)[16,17]. According to Grube et al.[16], the fact that polymorphism in the *TLR5* gene has been associated with the development of IA strongly suggests that bronchial or pulmonary epithelial lesions are mainly responsible for immune response dysregulation by *Aspergillus* spp. In addition, epithelial cell homeostasis may be defective due to increased epithelial apoptosis, thereby compromising defenses against the fungus.

TABLE 2: Summary of studies published in recent years about the genetic association of genes encoding the Toll-like receptors with susceptibility to aspergillosis.

Gene	SNP(s)	Chromosome Location	Nucleotide / Amino Acid Exchange	Population	Cases	Controls	Odds ratio (CI 95%)	*p* Value	Ref
TLR1	rs4833095	4p14	239G>C	Italy	112	277	0.58 (0.36-0.95)	0.029	Smith et al.[21]
TLR1	rs4833095	4q14	239G>C	Netherlands	43	61	1.02 (0.53-1.96)	0.096	Boer et al.[19]
TLR1	rs5743611	4q14	743A>G	Netherlands	42	59	1.10 (0.39-3.08)	0.86	Boer et al.[19]
TLR2	rs5743708	4q31.3	Arg753Gln	United Kingdom	40	80	0.795 (0.151-4.150)	1.000	Carvalho et al.[14]
TLR2	rs5743708	4q31.3	Arg753Gln	Germany	41	109	-	0.16	Grube et al.[13]
TLR3	rs3775296	4q35.1	95C/A	Italy	42	147	2.41(1.27-4,58)	0.007	Carvalho et al.[20]
TLR4	rs4986790	9q33.1	Asp299Gly	United Kingdom	40	80	3.462 (1.477-8.110)	0,03	Carvalho et al.[14]
TLR4	rs4986790	9q33.1	Asp299Gly	Germany	41	107	-	0,13	Grube et al.[13]
TLR4	rs4986791	9q33.1	Thr399Ile	Germany	41	109	-	0,15	Grube et al.[13]
TLR4	rs4986791	9q33.1	1363C>T	Netherlands	42	61	2.81 (0.91-8.70)	0.06	Boer et al.[19]
TLR4	rs4986790	9q33.1	1063A>G	Netherlands	43	61	4.33 (1.33-14,1)	0.01	Boer et al.[19]
TLR5	rs5744168	1q41	Arg392Ter	Germany	41	109	3.285 (1.20-8.99)	0.007	Grube et al.[13]
TLR6	rs5743810	4p14	745C>T	Netherlands	42	59	1.14 (0.65-2.00)	0.67	Boer et al.[19]
TLR9	rs5743836	3q21.2	T-123C	United Kingdom	40	80	0.927 (0.362-2.373)	0.43	Carvalho et al.[14]
TLR9	rs352140	3q21.2	P545P	Germany	41	110	-	0.19	Grube et al.[13]

SNP: Single nucleotide polymorphism; **Ref:** References, **CI:** Confidence interval.

TABLE 3: Summary of studies published about the association of *CLEC7A* polymorphisms with susceptibility to aspergillosis.

Gene	SNP(s)	Chromosome Location	Nucleotide / Amino Acid Exchange	Population	Cases	Controls	Odds ratio (CI 95%)	*p* Value	Ref
CLEC7A	rs16910526	12p13.2	aY238X	Italy	39	166	3.39(1.5-10.0) (D+R) 2.5(1.0-6.5) (D)	0.005 (D+R) 0.05(D)	Cunha et al.[1]
CLEC7A	rs16910526	12p13.2	aY238X	Belgium	71	108	1.79(0.77-4.19)	0.017	Chai et al.[18]
CLEC7A	rs7309123	12p13.2	-	England	57	125	4.91(1.52-15.89)	0.05	Sainz et al.[11]
CLEC7A	rs7309123	12p13.2	-	Spain	112	279	0.59(0.35-0.99)	0.046	Smith et al.[21]
CLEC7A	rs3901533	12p13.2	-	England	57	125	5.59(1.37-22.77)	0.012	Sainz et al.[11]

SNP: Single nucleotide polymorphism; **Ref:** References; **CI:** Confidence interval; **D:** Donor; **R:** Receiver.

The polymorphism (rs5744168) of the *TLR5* gene has already been associated with a greater susceptibility to pneumonia, where the receptor recognizes the flagellin of the bacterium *Legionella pneumophilla*. Another study conducted in a Jewish population reported that the *TLR5* stop codon provides protection against Crohn's disease[22]. Several studies have also linked *TLR9* genetic polymorphisms with various pathologies such as cervical cancer, lupus nephritis, and cerebral malaria. However, there are few studies that describe the association of gene polymorphisms with IA, and it is necessary to carry out larger studies to confirm these associations[22].

Studies have found that defective dectin-1 receptor functioning results from a stop codon polymorphism and could potentially increase susceptibility to IA[1,14,21,23,24]. Cunha et al.[1] confirmed this finding when evaluating both donors and bone marrow transplant recipients (OR = 1.5, 95% CI = 0.5–5.0, p = 0.005). They revealed that dectin-1 receptor variation is a predisposing factor for IA in high risk patients. This confirms the suspicion that such a receptor has a role in controlling resistance and immune tolerance to *Aspergillus* spp.

Another study confirming the association between dectin-1 variation and susceptibility to IA was performed by Sainz et al.[14], but the SNP rs7309123 was used. The level of significance for the association was similar to the previous study (OR = 4.91, 95% CI = 1.52–15.9, p = 0.05)[14]. They also showed an increase in the number of galactomannan-positive patients with this polymorphism.

The association between variability in the gene encoding dectin-1 and IA was also found in a study by Chai et al.[21] (OR = 1.79, 95% CI = 0.77–4.19, p = 0.017), which described that the studied polymorphism increased the susceptibility to IA. Similar results were observed by Smith et al.[24], who investigated the genetic association of 112 biologically plausible patients with IA and 279 healthy controls. The expression of genes in monocytes from IA patients and controls was investigated before and during stimulation with *Aspergillus fumigatus*. From the tests performed, an association between the *CLEC7A* SNP rs7309123 with IA was observed. However, according to the authors, a limitation of the study is that the investigated population was Caucasian, which necessitates additional studies in other ethnic groups to determine generalized susceptibility.

Several polymorphisms in genes encoding components of innate immunity have recently been reported to increase susceptibility to *Aspergillus* infections[13-15,17,18,21]. According to Chai et al.[21], the possibility of a patient presenting more than one polymorphism and consequently having a high susceptibility of developing IA should not be disregarded.

According to the studies reviewed here, there is a significant association between genetic polymorphisms and the development of IA. However, broader and larger studies regarding each of the SNPs presented should be performed for a better evaluation and, consequently, more reliable results. Such studies are important because the identification of concrete genetic polymorphisms associated with IA will enable the identification of patients at high risk of developing the pathology. As a result, efficient diagnostic procedures can be developed using the polymerase chain reaction technique.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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