# Risk factors and new markers of pulmonary fungal infection

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# Chapter 6

## THE Y238X STOP CODON POLYMORPHISM IN THE HUMAN BETA-GLUCAN RECEPTOR DECTIN-1 AND SUSCEPTIBILITY TO INVASIVE ASPERGILLOSIS

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## Abstract

**Background:** Dectin-1 is the major receptor for fungal beta-glucans on myeloid cells. We investigated whether defective Dectin-1 receptor function, due to the early stop codon polymorphism Y238X enhances susceptibility to invasive aspergillosis (IA) in at-risk patients.
**Methods:** Association of the Dectin-1 Y238X polymorphism with occurrence of IA was evaluated in a cohort of 71 patients who developed IA post hematopoietic stem cell transplantation (HSCT), and in a separate cohort of 21 non-HSCT patients who had IA post-chemotherapy. The control group consisted of 108 patients who had undergone HSCT. Presence of the Y238X polymorphism was linked with the occurrence and clinical course of IA. Functional studies were performed to investigate the consequences of the Y238X Dectin-1 polymorphism.
**Results:** In HSCT recipients, heterozygosity for the Y238X polymorphism showed a trend towards IA susceptibility (odds ratio 1.79, 95% confidence interval 0.77-4.19, p=0.17). Possession of Y238X polymorphism did not influence the clinical course of IA. The Y238X allele frequency was higher in non-HSCT patients with IA (19.0%) as compared to HSCT patient/donor cohort and the healthy population (range 6.9%-7.7%, $p<0.05$). Functional assays revealed that human peripheral blood mononuclear cells with complete defect in Dectin-1 function due to Y238X responded less efficiently to Aspergillus. However, macrophages showed an adequate response to Aspergillus despite deficient Dectin-1 function.
**Conclusions:** Dectin-1 Y238X heterozygosity has a moderate influence on susceptibility to IA. This is partly attributable to redundancy inherent in the innate immune system. The Y238X polymorphism may be important in susceptible non-HSCT patients. Larger studies are needed to confirm these findings.

## Introduction

Invasive fungal infections (IFI) remain a major cause of morbidity and mortality in immunocompromised patients, of which invasive aspergillosis (IA) is emerging as the most common IFI [1, 2]. Susceptibility and host response to fungal infection is largely determined by the immune status of the host, its ability to recognize the pathogen and to respond appropriately [3]. The mechanism responsible for this recognition is represented by pattern recognition receptors (PRRs) which include the family of Toll-like receptors (TLR) and C-type lectin receptors (CLR) [4]. Dectin-1 is a C-type lectin receptor present on human immune cells e.g. macrophages and monocytes. It recognizes the β-1,3-glucan motif present on the cell walls of Candida and Aspergillus species, and mediates host immune response to these fungal pathogens [5].

Recently, we described a functional single nucleotide polymorphism (SNP) in Dectin-1 (Y238X, rs16910526) leading to an early stop codon which resulted in loss of the last ten amino acids of the carbohydrate-recognition domain of the Dectin-1 receptor. Subsequently, this resulted in diminished expression of the Dectin-1 receptor on immune cells and its inability to bind β-glucan, leading to defective production of proinflammatory cytokines [6]. Clinically, this polymorphism was found to be associated with colonization with Candida spp. in hematopoietic stem cell transplantation (HSCT) recipients [7], as well as recurrent mucocutaneous fungal infection in a Dutch family [6].

Results from in-vitro and murine models have shown that Dectin-1 is pivotal in host defense against Aspergillus infection [8-10]. However, no data is available from human studies to validate these findings. Hence, we aimed to investigate the clinical relevance of the Dectin-1 early stop codon polymorphism for the susceptibility and outcome of IA in a cohort of patients with underlying hematological disorders.



## Patients and Methods

### Patient population

Ninety-two patients of Dutch-Flemish ancestry with underlying hematological diseases in which IA was diagnosed were enrolled from 3 academic hospitals: Leiden University Medical Center, Radboud University Nijmegen Medical Center, both in the Netherlands, and University Hospitals Leuven, Belgium between May 1996 to July 2009. Of these 92 patients, 71 developed IA following allogenic HSCT while 21 other patients had IA after receiving chemotherapy but without undergoing HSCT. Invasive aspergillosis had been diagnosed as either proven or probable IA as per current European Organization for Research and Treatment of Cancer/Mycology Study Group (EORTC/MSG) criteria [11]. One hundred and eight patients with comparable underlying disorders who underwent HSCT but did not develop IA were

enrolled as controls for the HSCT patients. All HSCT IA patients and the control patients had undergone T cell-depleted allogenic HSCT. The clinical characteristics of the HSCT patients and controls are summarized in Table 1.

Of the 21 non-HSCT patients who developed IA following chemotherapy, 18 patients had acute myeloid leukemia, except for one case each of acute lymphocytic leukemia, multiple myeloma and aplastic anaemia. The median age was 50 years, (interquartile range [IQR] 40-61), and 13 of the 21 subjects (62%) were males. Seventeen of the 21 cases (81%) had probable IA, while proven IA was diagnosed in the remaining 4 patients. Prolonged neutropenia (defined as absolute neutrophil count < 500 cells/mm3 for more than 14 days prior diagnosis of IA) was present in 9/21 cases (43%).

None of the patients or controls in this study received prior mould-active anti-fungal prophylaxis [12, 13]. DNA was obtained from patients following informed consent as required by the ethical committee of each respective institution. For all HSCT cases, DNA was obtained from both recipients and their respective donors prior to transplantation.

**Table 1.** Patient Demographics and Clinical Characteristics.

| Variable | Patients with IA | Patients without IA | p-value |
|---|---|---|---|
| **Total No.** | **71** | **108** | |
| **Sex ratio m/f** | 47/24 | 71/31 | 0.95 |
| **Age (median, IQR)** | 47 (40-57) | 48 (40-56) | 0.84 |
| **Hematological disease n (%)** | | | |
| AML | 24 (34) | 39 (36) | |
| CML | 11 (16) | 18 (17) | |
| ALL | 10 (14) | 12 (11) | |
| NHL | 9 (13) | 14 (13) | |
| Aplastic anemia | 2 (3) | 1 (1) | |
| CLL | 3 (4) | 7 (7) | |
| Multiple myeloma | 6 (8) | 1 (1) | |
| MDS | 6 (8) | 16 (15) | |
| **EORTC/MSG 2008 classification** | | | |
| Proven IA | 15 | - | |
| Probable IA | 56 | - | |
| **Prolonged neutropenia** | 31/71 | 40/108 | 0.46 |
| **Site of IA n (%)** | | | |
| Pulmonary | 68 (96) | - | |
| Extra-pulmonary | 3 (4) | - | |
| **GVHD** | 34/71 | 58/104† | 0.36 |

The median period of follow-up was 8.4 months (range 0.1-170.7) for patients with invasive aspergillosis and 59.9 months (range 0.4-163.9) for control patients. IA denotes invasive aspergillosis; IQR : interquartile range, HSCT: hematopoietic stem cell transplantation, AML : acute myeloid leukemia, CML : chronic myeloid leukemia, ALL: acute lymphocytic leukemia, NHL : non-Hodgekin's lymphoma, CLL : chronic lymphocytic leukemia, MDS :myelodysplastic syndrome, Prolonged neutropenia was defined as absolute neutrophil count < 500 cells/$mm^3$ for a period of more than 14 days prior diagnosis of IA, GVHD : graft-versus-host disease. †GVHD data was not available for 4 control patients. P-values were calculated by student-t test for continuous- and Pearson-chi-square test for binary data.

### Genotyping for Dectin-1 Y238X Polymorphism

The Y238X SNP (rs16910526) in exon 6 is the only known exonic polymorphism in the Dectin-1 gene in Caucasian populations [7]. Genotyping for the presence of the Y238X polymorphism was performed using the TaqMan SNP assay C_33748481_10 on the 7300 ABI Real-Time polymerase chain reaction system (Applied Biosystems).

### Cytokine stimulation assays

Cytokine profiling was performed to ascertain the functional consequence of the Dectin-1 Y238X polymorphism. The isolation of peripheral blood mononuclear cells (PBMC) and differentiation of monocyte-derived macrophages (MDM) from study subjects were performed as previously described [14]. The cells were stimulated with live and heat-killed conidia, as well as with heat-killed hyphae of a well-characterized Aspergillus fumigatus clinical strain, V05-27 [15]. Where indicated, Candida albicans blastoconidia belonging to strain ATCC MYA-3573 (UC820) [16] and particulate β-glucan (courtesy of Dr David Williams, University of Tennessee) were used as control stimuli. The supernatants were collected after 24 h of incubation at 37oC and stored at -20oC until cytokine assay. Interleukin-6 (IL-6) and tumor necrosis factor alpha (TNF-α) concentrations were measured by commercial sandwich ELISA kits (Pelikine Compact, CLB, Amsterdam, Netherlands and R&D Systems respectively) according to manufacturers' instructions.

### Flow cytometry

Freshly isolated human PBMC were incubated with 5 μg/ml murine anti-Dectin-1 mAb 259931 (R&D Systems, Minneapolis, MN) or mouse IgG2b isotype control in RPMI 1640 (supplemented with 2% human serum) followed by allophycocyanin-conjugated goat anti-mouse Ab (BD Pharmingen, San Diego, CA). Monocytes were labeled with anti-CD14-PE (Pelicluster, Sanquin, Amsterdam, The Netherlands) and Dectin-1 expression on CD14+ cells was determined by flow cytometry (FACScalibur, BD Biosciences). Detection of Dectin-1 receptor surface expression on MDM was performed as described above. Surface mannose receptor (MR), TLR2 and TLR4 expression was determined using anti-MR-FITC (R&D Systems), anti-TLR2-FITC and anti-TLR4-PE (both from eBioscience, San Diego, CA) in addition to their respective isotype controls.

### Statistical Analysis

Genotype frequencies were compared between groups by Fisher's exact- and Pearson-chi-square tests. Odds ratio (OR) and 95% confidence interval (95%CI) were calculated for the

presence (homozygous or heterozygous) or absence (homozygous wild type allele) of the Dectin-1 Y238X polymorphism. Multivariate adjustments for neutropenia and development of graft-versus-host disease (GVHD) were made where appropriate. The influence of the variant Dectin-1 SNP on the clinical course of disease i.e. day from HSCT or start of chemotherapy to the day of diagnosis was assessed by Kaplan-Meier analysis (logrank test). Likewise, associations with presence of the polymorphism and time from IA diagnosis to death were assessed. The cytokine data was presented as mean + standard error of the mean (SEM). Differences in cytokine production were assessed by using Student's t test. A p-value of $<0.05$ was considered significant. The SPSS version 17.0 statistical software package for Windows was used to perform the calculations.

## Results

### Dectin-1 Y238X Polymorphism in IA Patients and Controls

Following HSCT, the primary immune cells of the recipient will eventually assume genotype of the donor after successful engraftment. Hence presence of the Dectin-1 Y238X SNP was determined in all patients and HSCT donors. The genotype frequencies of the study cohort were in Hardy Weinberg equilibrium. Thirteen of the 71 patients who developed IA post HSCT (18.3%) and 12 of 108 control patients (11.1%) had the Dectin-1 Y238X SNP. All these individuals were heterozygous for the SNP. Possession of the Y238X polymorphism was only associated with a limited trend towards IA susceptibility and this did not reach statistical significance (odds ratio [OR] 1.79, 95% confidence interval [CI] 0.77-4.19, p=0.17), see Table 2a. Following multivariate adjustment for neutropenia and GVHD, the adjusted OR was 1.70, 95% CI 0.72-4.00, p=0.22. Donor genotype did not influence risk of IA in the recipient. Likewise, simultaneous possession of Y238X in both HSCT donor-recipient pair did not increase susceptibility to IA.

In addition, the Dectin-1 Y238X SNP was found in 7 of the 21 non-HSCT patients (33.3%) who developed IA following immunosuppressive chemotherapy, of which one individual was homozygous for the Y238X polymorphism. Given the limited case patients in this non-HSCT cohort, we opted to compare the allelic frequencies of Dectin-1 Y238X variant against the following patient cohorts/healthy populations: (1) the HSCT patients with and without IA in this study who had similar underlying hematological diseases (2) the healthy HSCT donors (in this study) and (3) healthy population of comparable Dutch ancestry [6] (Table 2b). The allele frequency of the Y238X SNP was significantly elevated in the non-HSCT IA patients (19.0%) as compared to HSCT patients (7.0%, p=0.01), healthy HSCT donors (7.7%, p=0.04) and Dutch population (6.9%, p=0.02).

**Table 2a.** Incidence of Dectin-1 Y238X variant in HSCT patients/donors between IA cases and controls.

| Study Cohort | Cases Dectin-1 Y238X variant present n/N | Controls Dectin-1 Y238X variant present n/N | Univariate OR (95%CI) | p-value |
|---|---|---|---|---|
| **HSCT IA recipients vs control recipients** | 13/71 | 12/108 | 1.79 (0.77-4.19) | 0.17 |
| **HSCT donors to IA patients vs control donors†** | 10/68 | 17/107 | 0.91 (0.39-2.13) | 0.83 |
| **Presence of Y238X in both HSCT recipient & donor†** | 5/68 | 8/107 | 0.98 (0.27-2.80) | 0.98 |

† DNA belonging to HSCT donors of 3 IA cases and 1 control case were unavailable for genotyping. IA denotes invasive aspergillosis; HSCT: hematopoietic stem cell transplantation; OR: odds ratio; 95%CI: 95% confidence interval. P-values were obtained by Pearson-chi-square test.

**Table 2b.** Comparison of allele frequencies of Dectin-1 Y238X variant in susceptible patient cohorts and healthy populations.

| Study Cohort: | Non-HSCT patients with IA | HSCT patients (with- and without IA) | Healthy HSCT donors | Corresponding Healthy Population # |
|---|---|---|---|---|
| **Dectin-1 Y238X variant‡** | 7/21 | 25/179 | 27/175 | 19/138 |
| **Allele frequency** | 19.0%π | 7.0% | 7.7% | 6.9% |

IA: invasive aspergillosis. ‡: All individuals were heterozygous for the Y238X Dectin-1 polymorphism except for one individual in the Non-HSCT group who was homozygous. #: Dutch healthy population [6]. π : Frequency of the allele frequency was significantly higher as compared to the all three other populations (Fishers exact test: $p<0.05$), see text for details.



## Influence of Dectin-1 Polymorphism on Clinical Course of IA

In addition to its effect on the susceptibility to IA, we assessed whether the presence of the Dectin-1 variant gene might influence clinical course during IA. Kaplan-Meier analysis did not reveal a difference in time-to-development of IA from HSCT between recipients or their donors bearing either the wild-type (WT) or variant allele (Figures 1a &b, p=0.94 and p=0.88 respectively). There was no difference in survival (time to death following diagnosis of IA) consequent to having the WT or variant Dectin-1 allele in both patients and donors (Figures 2 a & b, p-logrank 0.83 and 0.99 respectively).

**Figure 1.** Time to diagnosis of invasive aspergillosis from start of HSCT conditioning/chemotherapy

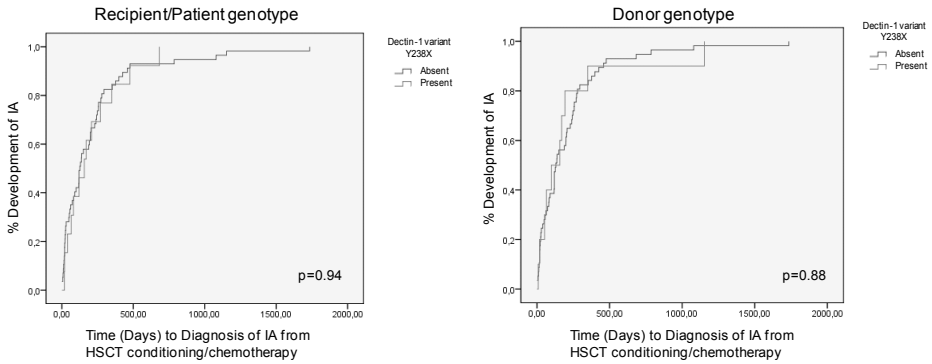
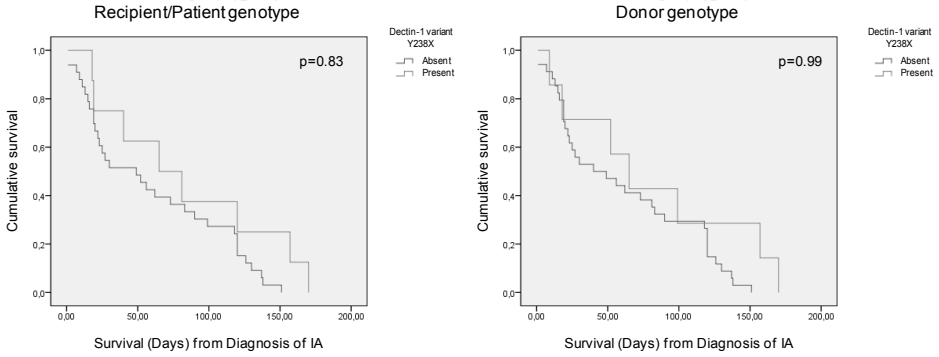


**Figure 2.** Patient survival following diagnosis of invasive aspergillosis




## Functional Consequences of the Dectin-1 Y238X Polymorphism

Functional assays were performed to attempt to find a mechanistic explanation on the limited influence of Dectin-1 on susceptibility to IA. To fully elucidate the phenotypic effects of the Dectin-1 Y238X polymorphism, we had used Dectin-1- deficient PBMC and differentiated MDM from two siblings whom we have characterized as being homozygous for the variant Dectin-1 allele [6], and from healthy control subjects who were WT for the Dectin-1 gene.

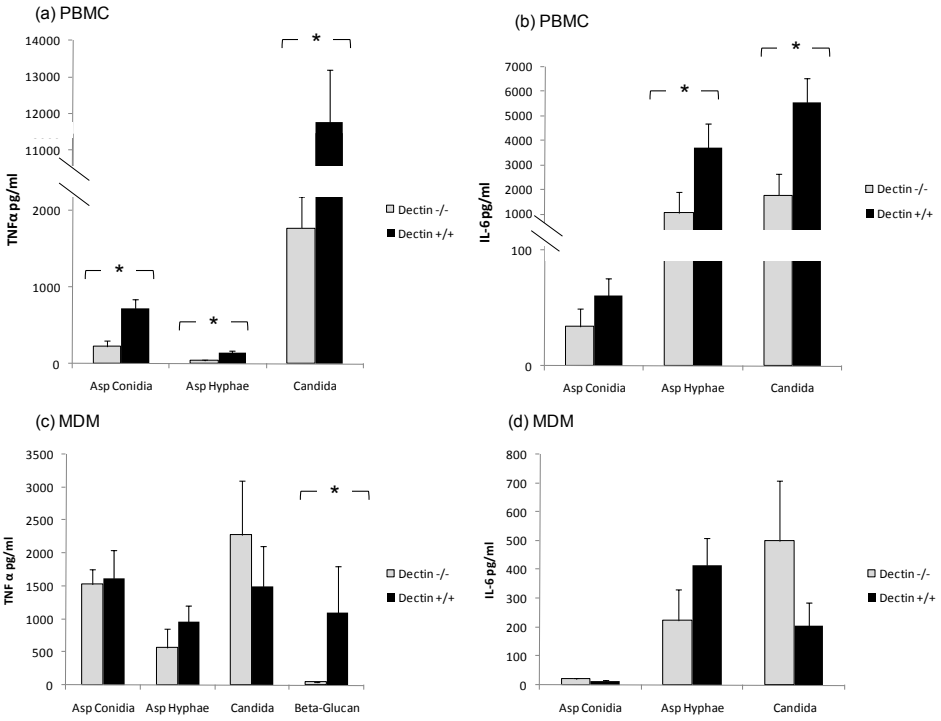
## Cytokine stimulation

We assessed the capacity of the immune cells to respond to the various stimuli. In PBMC, homozygosity for Dectin-1 Y238X resulted in marked reduction of proinflammatory TNF-α and IL-6 production in response to heat-killed A. fumigatus hyphae, C. albicans blastoconidia and live A. fumigatus conidia as would have been anticipated given the key role that the Dectin-1 receptor is known to play in recognition of fungal cell wall β-glucan (Figure 3a & b) [7, 9]. In the MDM, however, there were no significant differences in proinflammatory cytokine responses between subjects who were homozygous or WT for Dectin-1. This was apparent for both live A. fumigatus conidia, as well as heat-killed hyphae (Figures 3c & d). As control, stimulation using β-glucan still failed to generate TNF-α response in the Dectin-1-deficient MDM in contrast to MDM containing the wild-type Dectin-1. Despite the intrinsic inability to signal via the Dectin-1 pathway in the Dectin-1-deficient MDM, the demonstrated ability of these cells to still respond to Aspergillus indicated the presence of alternative signaling pathways.

## Flow Cytometry

We demonstrated earlier that monocytes from individuals homozygous for Y238X polymorphism had diminished Dectin-1 receptor cell surface expression [7]. We further demonstrate

**Figure 3.** Functional assays using dectin -/- PBMC and MDM




**Legend:** Functional assays to assess consequence of the Dectin-1 Y238X polymorphism in response to live *Aspergillus fumigatus* conidia $1x10^7$/ml (Asp Conidia), heat-killed *A. fumigatus* hyphae $1x10^7$/ml (Asp hyphae), *Candida albicans* blastoconidia $1x10^6$/ml (*Candida*) and beta-glucan 20µg/ml. Tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6) responses of peripheral blood mononuclear cells (PBMC, Fig 3a &b) and monocyte-derived macrophages (MDM, Fig 3c & d) from 2 sibling homozygous for the Y238X polymorphism (Dectin -/-) [6] and 5 Dectin-1 wild-type controls (Dectin +/+) were assessed. Data was from 3 sets of experiments and presented as mean ± standard error of the mean (SEM). * denotes statistical significance $p < 0.05$ as determined by Student's t test.

here that MDM from these individuals also had deficient expression of the Dectin-1 receptor (Figures 4a & c) which is corroborated by the inability to respond to β-glucan as shown above. Besides Dectin-1, other PRRs such as the mannose receptor (MR), TLR2 and TLR4 participate in recognition of fungal ligands [5]. Of note, the MR is a distinct CLR commonly found mainly on macrophages, while TLR2 and TLR4 are ubiquitous on most immune cells including monocytes and macrophages. It is plausible that the host tissue macrophages recognize Aspergillus through these alternative PRRs, especially the MR pathway. This may circumvent deficiency in the Dectin-1 signaling pathway, and account for the normal cytokine production in Dectin-1 deficient MDM. To substantiate this, we showed that Dectin-1 deficient MDM had similar levels of expression of MR as normal cells (Figures 4b & d), and that TLR2 and TLR4 expression was normal (data not shown).

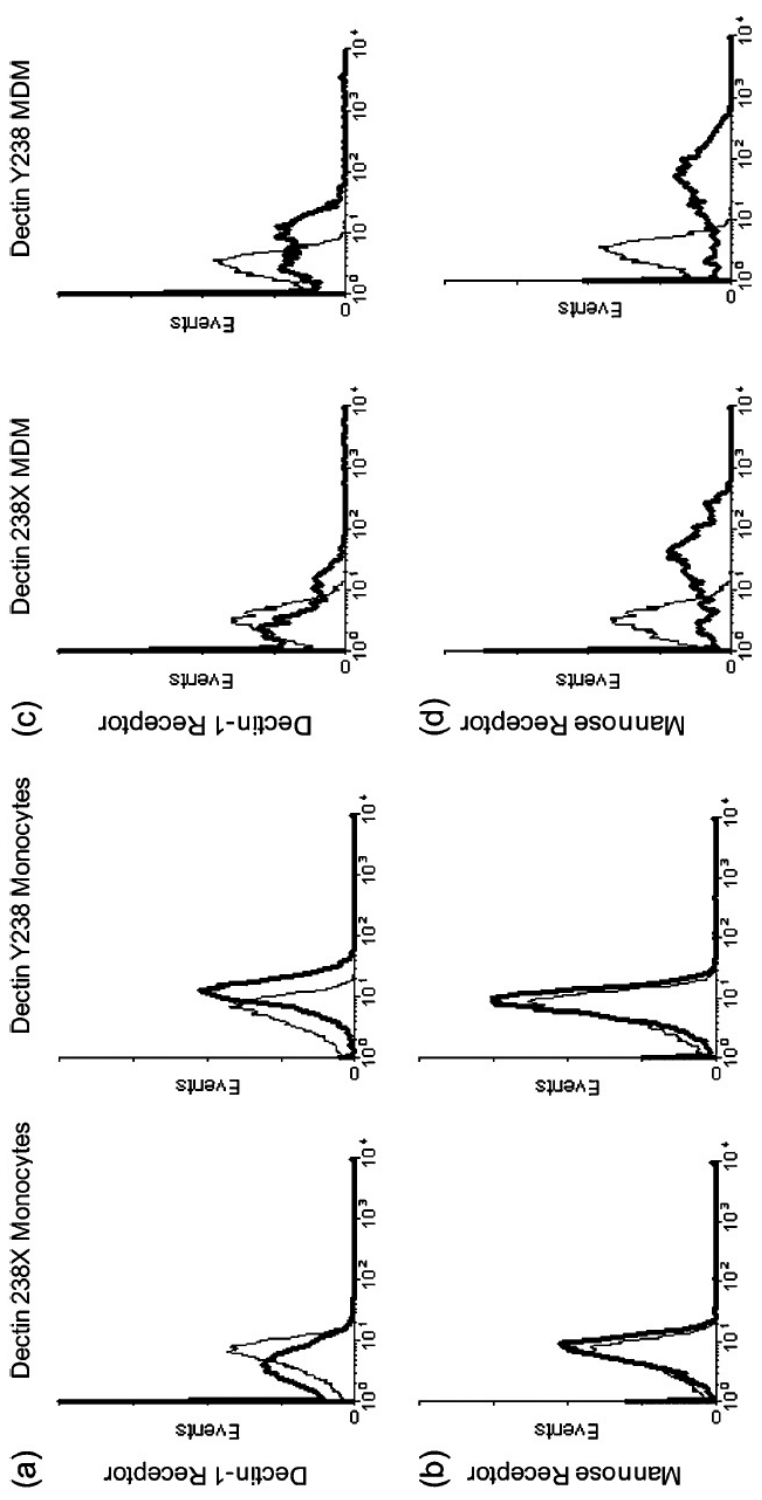



**Figure 4.** Flow cytometry on Dectin-/- monocytes and MDM

**Legend:** Representative flow cytometry analysis on surface staining of human Dectin-1 receptor and mannose receptor on Dectin -/- and Dectin +/+ CD14+ monocytes (Fig 4a & b) and MDM (Fig 4c & d). Thin lines represent the respective isotype controls.

## Discussion

Though much has been reported on the central role that Dectin-1 plays in host recognition of Aspergillus, this is based largely on findings from experimental murine models. In this study, however, we found that a defective function of Dectin-1 due to a premature stop codon polymorphism may potentially enhance susceptibility to IA in susceptible non-HSCT patients although the effect was moderate in the HSCT cohort and did not significantly alter the clinical course of the disease. In contrast to the observations on Dectin-1 obtained from in-vitro and mice models, the above clinical findings remain significant as they also highlight the system of redundancy inherent in the human innate immune system against invading pathogens like Aspergillus.

Recently, Dectin-1 has been recognized as being a pivotal PRR for the control of fungal infections [17] and specifically for anti-Aspergillus host defense [10]. In-vitro studies demonstrated the involvement of Dectin-1 in both TLR-dependent and TLR-independent anti-fungal responses [14, 18]. The clinical significance of Dectin-1 in mucosal candidiasis was highlighted recently in a study that described how defective Dectin-1 expression and function resulted in recurrent vulvovaginal candidiasis in a family of siblings who were homozygous for the Y238X polymorphism [6], while another study reported an increased incidence of oral and gastrointestinal Candida colonization in HSCT recipients heterozygous for the same variant gene [7].



Despite the in-vitro studies pointing out the importance of Dectin-1 as receptor for fungal β-glucans, the perceived importance of Dectin-1 for invasive mycosis in mice models had been debated. Contrary to findings of Taylor et al that Dectin-1 deficient mice showed increased susceptibility to disseminated candidiasis, another study by Saijo et al did not yield similar corresponding results using an independently developed Dectin-1 -/- mice strain [19, 20]. A later study showed that Dectin-1 had an important role in a murine model of invasive aspergillosis [10]. Nevertheless, it was also interesting to note that a family with siblings who had a complete deficiency of the Dectin-1 function did not report susceptibility to systemic fungal infections [6]. This suggests that although Dectin-1 has an unchallenged role as β-glucan receptor, in the in-vivo situation, alternative recognition pathways can initiate effective anti-fungal responses.

Patient studies remain crucial for the validation of the host defense mechanisms identified in in-vitro and experimental studies. To be at-risk for development of IA, a profoundly immunocompromised status consequent to immune-ablative chemotherapy, HSCT conditioning regimens or chronic corticosteroid treatment is obligate. In this study we found a markedly increased Y238X allele frequency of 19.0% in non-HSCT patients who developed IA post-chemotherapy as compared to other reference populations (range 6.9-7.7%). In concordance to earlier findings from a previous study which reported the Y238X allele frequency to be 6.9%

in the general Dutch population, our analysis of healthy donors to HSCT patients also yielded a comparable allele frequency of 7.7% (p=0.76). One consideration would have been whether the possession of the Y238X polymorphism could remotely be related to acquisition and progression of the underlying hematological disease state resulting in an over-represented allele frequency in the above non-HSCT IA cases. However, this was not the case as we had also determined that in the HSCT patients with similar predisposing hematological disorders, the allele frequency of Y238X was 7.0%; this was comparable to the above healthy populations.

The occurrence of IA in HSCT presents a challenge in studying genetic susceptibility as both donor and recipient genotype will invariably exert their influence on function of the immune cells post transplantation. Even after documented engraftment, it remains unresolved when chimerism is actually achieved at the level of the pulmonary macrophages which form the frontline against the invading Aspergillus. We found that Y238X status in the HSCT recipient was associated with a modest trend towards susceptibility to IA (OR 1.79, 95% CI 0.77-4.19, p=0.17). This was not accentuated following multivariate adjustment (adjusted OR 1.70, 95% CI 0.72-4.00, p=0.22). Also, donor Y238X status was found not to be an attributable factor. Simultaneous presence of the Dectin-1 Y238X variant in both donor-recipient pair did not further confer a dose-dependent effect on susceptibility to IA (Table 2a). Immune recognition and activation at the epithelial level is a key mechanism in host defense against invasive pathogens [21]. Post-HSCT, Dectin-1 expression on epithelial cells and pneumocytes remains as determined by recipient genotype in contrast to immune cells of myeloid origin. Hence this reasonably accounts for our finding that it was the recipient Dectin-1 Y238X status, rather than the donor, which had an influence on susceptibility to IA.

The stronger association observed in the non-HSCT cohort despite the smaller patient numbers may be because patient and treatment profiles are relatively more homogenous. Comparisons incorporating both cohorts, though, may be confounded as there remain inherent differences in treatment regimens (and possibly IA susceptibility) between non-HSCT and HSCT patients [22]. Nevertheless, the increased incidence of the Dectin-1 Y238X variant in non-HSCT IA patients, as well as its association towards IA susceptibility in HSCT recipients, suggest that heterozygosity for the Y238X SNP has a moderate association with acquisition of IA in at-risk patients. Recognizing the potential limitation of our finding - pertaining specifically to the smaller non-HSCT study cohort as well as the methodology employed for the sub-group analysis - further validation of this observation in a larger cohort of non-HSCT IA patients and controls is needed.

Our functional assays using cells isolated from individuals homozygous for the Dectin-1 Y238X polymorphism also shed light on why susceptibility to Aspergillus infection may be limited and clinical course of disease relatively unaltered despite reduced Dectin-1 receptor function. In contrast to the PBMC, Dectin-1 defective MDM had the capability to respond

with normal production of proinflammatory cytokines upon challenge with A. fumigatus. As pulmonary macrophages form the first line of defense against inhaled Aspergillus conidia, our findings highlight the capacity of macrophages to retain their response even with deficient Dectin-1 function probably lies in their capacity to engage alternative PRRs: MR, TLR2 and TLR4. These receptors are known to be involved in immune recognition of Aspergillus and antifungal host defense [23-26]. This ability to retain the capacity to respond to the pathogen in the absence of Dectin-1 underscores the redundancy that is inherent to the human antifungal host defense. On the other hand, the modest susceptibility to aspergillosis in patients bearing the Y238X polymorphism, coupled to the defective monocyte function, suggest an adjuvant yet essential role of infiltrating monocytes for host defense. In contrast to monocytes and macrophages, the main β-glucan receptor on neutrophils is complement receptor 3 [27]. Although neutrophils are important in anti-Aspergillus host defense [28], the Dectin-1 Y238X polymorphism does not affect these cells as neutrophil function was normal in individuals with homozygous Dectin-1 Y238X mutation [6].

Other polymorphisms in genes coding for components of the innate immunity have been recently reported to increase susceptibility to Aspergillus infections: TLR1, TLR4, TLR6 and IL-10 promoter [29-34]. In all of the above studies, like ours, the polymorphism of interest was studied in isolation and not in association with each other. It is tempting to consider that the concomitant presence of two or more of these polymorphisms in a patient may further enhance the risk profile to IA.

In conclusion, in the present study we report that the Dectin-1 Y238X polymorphism was associated with a moderate increase in susceptibility to IA, particularly in non-HSCT immunocompromised patients. Additional studies are needed to validate these findings, yet these data provide novel insight in human host defense during invasive aspergillosis.



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