

CYTOKINE GENE POLYMORPHISMS IN PIGEON BREEDER'S DISEASE EXPRESSION

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ABSTRACT. *Background:* Exaggerated immunological response to repeated inhalation of organic or chemical dusts may lead to Hypersensitivity Pneumonitis among sensitized individuals. Only a few exposed individuals became ill and disease expression pattern is highly variable which suggest that genetic factors may play a role. *Aim:* To investigate interferon (IFN)- γ , tumour necrosis factor (TNF)- α , interleukin (IL)-6, transforming growth factor (TGF)- β , and IL-10 gene polymorphisms in a cohort of pigeon breeder's disease (PBD) patients in comparison with exposed but healthy controls and the association with different patterns of disease. *Methods:* We evaluated 40 PBD patients and 70 exposed controls. IFN- γ , TNF- α , IL-6, TGF- β , and IL-10 polymorphisms were determined by polymerase chain reaction–sequence specific primer amplification. *Results:* Polymorphism analysis of IFN- γ , TNF- α , IL-6, TGF- β , and IL-10 genotypes and allele frequencies showed no differences between patients and controls. IFN- γ T/T genotype frequency was increased among patients with chronic presentation (RR=2.33, p=0.047) compared with those with acute/subacute presentation. Also, chronic presenting patients had an increased frequency of IFN- γ T allele (50% vs 22.5%, RR=1.76, p=0.011). No differences were found in TNF- α , IL-6, TGF- β , and IL-10 genotypes neither allelic frequencies between both groups of patients. IL-6 C/C genotype was more frequent in patients who showed chronic evolution (RR=2.54, p=0.017), when comparing with patients with disease resolution. *Conclusion:* IFN- γ T/T and the IL-6 C/C genotypes seem to play a role in HP expression due to avian exposure, as their frequencies are increased in chronic presentations or in those with chronic evolution one year after the initial diagnosis, respectively. (*Sarcoidosis Vasc Diffuse Lung Dis* 2020; 37 (3): e2020004)

KEY WORDS: Pigeon breeder's disease, hypersensitivity pneumonitis, cytokines, genetic polymorphisms

INTRODUCTION

Hypersensitivity pneumonitis (HP) is an immunologically mediated diffuse lung disease (DLD)

caused by repeated inhalation of organic and/or chemical dusts by sensitized individuals (1–3). One of the most common forms of HP is pigeon breeder's disease which results from inhalation of avian proteins (1,2). The acute phase of the disease is characterized by specific antibody reaction to inhaled antigens and a putative local immune complexes deposition within the alveolar walls, triggering a neutrophil-mediated inflammatory response (4–6). Afterwards, subacute and chronic phases may devel-

Received: 26 February 2020

Accepted after revision: 9 September 2020

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op (7). These are proposed to be mediated by macrophages (8), cytotoxic (9–11) and type 1 T helper (Th1) (12) lymphocytes with consequent increasing of several cellular immunity-related cytokines, such as interferon-gamma (IFN- γ), tumour necrosis factor (TNF)- α , interleukin (IL)-2, along with other pro-inflammatory cytokines such as IL-6 and IL-8 (13,14). Thus, a Th1-driven delayed-type hypersensitivity may eventually result in granuloma formation and, later, pulmonary fibrosis (15–17). On the other hand, Th1 activity may be under the regulatory influence of other cytokines, such as transforming growth factor (TGF)- β and IL-10 (14,18,19).

Although HP diagnostic criteria of the disease have been well discussed and proposed based on clinical, radiologic and immunological features (20,21), there are still some unexplained features, namely the reason why only a small proportion of antigen exposed individuals develop the disease and individuals with similar exposure levels have different patterns of disease expression and/or evolution. These observations suggest that, similarly to other granulomatous DLD such as sarcoidosis, individual genetic factors may play a role in susceptibility and variability of HP expression (22–26). In this sense, cytokines, while essential for cellular communication and also having a relevant role in immune response modulation, may show individual variability in tissue expression and/or circulating levels (25). Several studies have shown a connection between some cytokine gene polymorphisms (TNF- α , IFN- γ , IL-6, TGF- β , and IL-10) and the level of expression of those cytokines (25–32).

Regarding HP, a limited number of studies relating cytokine genetic polymorphisms with disease development have been performed, with controversial results (25,29,33–35). So, we investigated IFN- γ , TNF- α , IL-6, TGF- β , and IL-10 gene polymorphisms among Portuguese patients with pigeon breeder's disease, comparatively with exposed but healthy controls. Furthermore, we compared the same genetic polymorphisms among pigeon breeder's disease patients with different patterns of disease expression and evolution.

MATERIAL AND METHODS

Patients' selection

HP patients (n=40) were prospectively and consecutively recruited from the Centro Hospitalar e Universitário de São João (CHUSJ), a University Hospital in Porto, Portugal. All patients had pigeon breeder's disease (PBD) and were native Portuguese, Caucasians and unrelated. The diagnosis PBD was established by a multidisciplinary team (MDT) (36,37) according to the following criteria (38): 1) avian exposure; 2) clinical and imagiological features of HP and 3) bronchoalveolar lavage (BAL) lymphocytes > 40%. These criteria were not totally met in eleven participants, in whom HP diagnosis required surgical lung biopsy. HP patients were followed-up in our center for 6.1 ± 3.6 years. Disease presentation was classified in acute, subacute or chronic depending on clinical features. Acute presentation was defined as development of influenza-like symptoms such as chills, fever, sweating and myalgias commonly associated to respiratory symptoms such as dyspnea, cough, chest tightness and bibasilar crackles in a few hours after antigen exposure and lasting from hours to days. Subacute presentation consisted in a gradual onset of respiratory symptoms, such as cough and dyspnea, over several days to weeks, which may progress and lead to hospitalization. Chronic presentation was defined as insidious onset of progressive exertional dyspnea and dry cough, frequently concomitant fatigue and weight loss for several months (39,40). Since there is evidence that subacute presentation is difficult to individualize and some authors propose an alternative classification in two phenotypes (38,41–43), for subgroup analysis and concerning disease presentation, we evaluated two subgroups of HP patients: acute/subacute *versus* chronic. A consensual time period since diagnosis in which HP evolution is definitely considered to have a chronic evolution is not established (38); thus, in the present study, patients who evolved to chronicity presented persistence of respiratory symptoms and/or irreversible lung fibrosis after one year of the initial diagnosis. On the other hand, disease resolution was considered when respiratory and/or systemic symptoms disappeared and there was no clinical, functional or imagiological evidence of interstitial lung disease.

All patients underwent the same HP management protocol from our center. This protocol include antigen avoidance as the first step and, if there is worsening or even persistence of symptoms and/or imagiological findings, oral corticosteroids were initiated (usually prednisolone, 0.5 mg/kg), followed by progressive tapering according to clinical evolution (44). When disease is progressive despite corticosteroids or if required for long periods, aiming to a steroid-sparing effect, other immunosuppressants are considered, namely azathioprine (2 mg/kg, with a maximum dose of 150 mg/day) or mycophenolate mofetil (1500–2000 mg/day) (45,46).

Our control group consisted of 70 exposed, unrelated, and healthy bone marrow donors, all from the same region and ethnic background of the included patients. Exposure was determined by direct questionnaire. These controls had a regular medical follow-up in their general practitioner, without any clinical or imagiological suspicion of respiratory disease. Written informed consent was obtained of all participants and the study had approval from Ethics Committee of CHUSJ.

DNA extraction

Genomic DNA was isolated from anticoagulated venous blood samples by phenol precipitation followed by digestion with proteinase K using Puregene DNA isolation Kit (Gentra Systems, Minneapolis MN).

Cytokine genotyping

Cytokine genotypes were determined by polymerase chain reaction (PCR)–sequence specific primer amplification using the Cytokine Genotyping Tray (One Lambda, Inc., Canoga Park, CA). Single-nucleotide polymorphisms for five cytokines were analyzed (47,48); the positions of the polymorphic sites tested were as follow (47,48): –174 base pair (BP) promoter/enhancer region of IL-6 (31); –1082, –819, and –592 bp promoter region of IL-10 (49,50); –308 bp promoter/enhancer region of TNF- α (49,51); CA dinucleotide repeats in intron 1 of IFN- γ (49,52); and codons 10 and 25 of the signal sequence of TGF- β (49,53,54). All typing analyses described above included positive and negative controls. All PCR products were fractionated elec-

trophoretically in 2% agarose-gel and visualized by ethidium bromide staining and ultraviolet light.

Statistical analysis

Genotype and allele frequencies were determined by direct counting. Goodness of fit of controls' allele frequencies to Hardy-Weinberg equilibrium were analyzed using a chi-square test. Differences between groups were evaluated through chi-square or Fisher exact tests, when appropriate. Odds ratios (OR) or Relative Risks (RR) and their respective 95% confidence intervals (95% CI) were also calculated. Lung functional and BALF parameters in patients' genotypes subgroups were compared through Kruskal-Wallis test. Two-sided P-values lower than 0.05 were considered statistically significant. All analysis was performed within R Studio, a computing environment for R programming language.

RESULTS

The included patients were older than the exposed healthy controls (48.2 \pm 15.4 *versus* 32.9 \pm 7.9 years-old; $p < 0.001$), although with a similar gender distribution (females 60% *versus* 65%, $p = 0.69$) and without statistically significant differences on the mean of exposure time (12.4 \pm 11.9 *versus* 8.9 \pm 7.3 years, $p = 0.10$). Demographic and clinical features of HP patients are shown in TABLE 1. Concerning disease classification, half of the patients had acute/subacute presentations while the other half had chronic forms. Resolution was documented in 52.6% and evolution to chronicity in 47.4% of patients. No significant differences on the treatment protocol (antigen avoidance with or without immunosuppression) were seen between subgroups (data not shown).

Allele distributions of IFN- γ (+874 T/A), TNF- α (-308 A/G), IL-6 (-174), TGF- β (codon 10 T/C, codon 25 C/G) and IL-10 (-592 C/A, -819 T/C, -1082 G/A), revealed no statistically significant deviations from Hardy-Weinberg equilibrium in the control group (data not shown).

Analysis of the studied gene polymorphisms encoding IFN- γ , TNF- α , IL-6, TGF- β and IL-10 and their allelic frequencies showed no statistically significant differences between HP patients and control groups (SUPPLEMENTARY TABLES 1 AND 2).

Table 1. Clinical and demographics characteristics of HP patients

Characteristics	HP Patients N=40
Gender,	
Female	24 (60%)
Male	16 (40%)
Age (years),	48.2±15.4
Exposure time (years)	12.4±11.9
Evolution (N=38)	
Chronicity	18 (47.4%)
Resolution	20 (52.6%)
Presentation	
Acute	11 (27.5%)
Sub-acute	9 (22.5%)
Chronic	20 (50.0%)
Lung functional parameters	
FVC % of predicted	71 (16.6-122)
FEV1 % of predicted	69 (19.2-118)
TLC % of predicted	76 (42-121.5)
DLCO % of predicted	58 (23-134)
DLCO/VA % of predicted	66 (39-168.9)
PaO2 mmHg	69 (45.4-101)
BAL features	
Cells x10⁻⁵/ml	4.6 (2-24)
Lymphocytes %	61.3 (24.4-87.6)
Neutrophils %	3.8 (0-33)
Eosinophils %	1 (0-11)
Mastocytes %	0.2 (0-2)
CD4+ lymphocytes %	39.05 (7.3-85.6)
CD8+ lymphocytes %	46.35 (7.1-80.1)
CD4+/CD8+ ratio	0.85 (0.12-12.1)

HP characteristics were described as n (%), mean ± SD or median (min-max) as appropriated.

HP Hypersensitivity pneumonitis; FVC – forced vital capacity; FEV1 – forced expiratory volume in one second; TLC – total lung capacity; DLCO – diffusing capacity of the lung for carbon monoxide; DLCO/VA – diffusing capacity of the lung for carbon monoxide divided by alveolar volume; PaO₂ – partial pressure of oxygen in arterial blood; BAL – bronchoalveolar lavage.

However, when comparing patients with acute/subacute with those with chronic presentations (TABLE 2), the IFN- γ T/T (high) genotype frequencies were significantly increased among the latter (RR=2.33, p=0.047). Conversely, although not statistically significant, patients with chronic presentation tended to have lower IFN- γ A/A (low) genotype frequencies (RR= 0.5, p=0.053). Regarding allelic frequencies analysis (TABLE 2), patients with chronic presentation had an increased frequency of IFN- γ T allele (50% vs 22.5%, RR=1.76, p=0.011), comparatively to those with acute/subacute presentations. No differences were found in TNF- α , IL-6, TGF- β , and IL-10 encoding genes neither in allelic frequencies between both groups of patients (data not shown).

Comparing patients who evolved to chronicity with those with disease resolution (TABLE 3), the IL-6 C/C (low) genotype was more frequent in those with chronic evolution (RR= 2.54, p=0.017). With regards to allelic frequencies (TABLE 3), IL-6 C allele tended to be more frequent among patients who evolved to chronicity (41.7% vs 22.5%, RR= 1.55, p=0.07). No differences were found in the studied IFN- γ , TNF- α , TGF- β , and IL-10 gene polymorphisms, neither in its allelic frequencies, between both groups (data not shown). Finally, lung functional parameters (namely forced vital capacity, forced expiratory volume in one second, total lung capacity, diffusing capacity of the lung for carbon monoxide, diffusing capacity of the lung, partial pressure of oxygen in arterial blood) and bronchoalveolar lavage (BAL) features (such as total and relative lymphocyte numbers, CD4+ and CD8+ lymphocytes) among patients' genotypes subgroups did not show any statistically significant differences (data not shown).

DISCUSSION

In this study, we found no statistically significant differences in genotype or allelic frequencies of single-nucleotide polymorphisms in the promoter/enhancer region of cytokine genes (IFN- γ , TNF- α , IL-6, TGF- β , and IL-10) in this Northern Portuguese cohort of patients with pigeon breeder's disease, comparatively with healthy exposed controls. However, further evaluating the different clinical patterns of disease, our results showed that patients with a chronic presentation have higher frequencies of IFN- γ T/T (high) genotype, comparatively with

Table 2. Comparison of IFN- γ genotypes and allele frequencies in patients with chronic (n=20) and with acute/subacute (n=20) presentations.

	Chronic	Acute/Subacute	RR	CI 95%	p value†
IFN-γ (+874)					
A/A (low)	5(25%)	11(55%)	0.5	0.23 ; 1.1	0.053
T/A (int)	10(50%)	9(45%)	1.11	0.6 ; 2.06	0.752
T/T (high)	5(25%)	0(0%)	2.33	1.59 ; 3.42	0.047‡
A allele	20(50%)	31(78%)	0.57	0.37 ; 0.87	0.011
T allele	20(50%)	9(23%)	1.76	1.16 ; 2.68	

RR Relative Risk; CI Confidence Interval; †Chi-square test; ‡ Fisher exact test

Table 3. Comparison of IL-6 genotypes and allele frequencies patients whose disease evolved to chronicity (n=18) and patients with disease resolution (n=20).

	Chronicity	Resolution	RR	CI 95%	p value†
IL6 (-174)					
C/C (low)	5(28%)	0(0%)	2.54	1.66 ; 3.88	0.017‡
G/C (high)	5(28%)	9(45%)	0.66	0.3 ; 1.46	0.272
G/G (high)	8(44%)	11(55%)	0.8	0.41 ; 1.58	0.516
C allele	15(42%)	9(23%)	1.55	0.98 ; 2.43	0.073
G allele	21(58%)	31(78%)	0.65	0.41 ; 1.02	

RR Relative Risk; CI Confidence Interval; †Chi-square test; ‡ Fisher exact test

those with acute/subacute presentations. Moreover, concerning disease evolution, the IL-6 C/C (low) genotype was more frequent in patients who evolved to chronicity than in those whose disease resolved. This supports the hypothesis that individual genetic factors may play a role in the variability of HP expression namely, in pigeon breeder's disease, functional gene polymorphisms of the IFN- γ and IL-6 cytokines.

Pigeon breeder's disease is a common HP form caused by avian antigens inhalation. Patients exposed to proteins of bird droppings and feathers, usually in a domestic environment, develop acute, subacute or chronic illnesses, and some of them evolve to diffuse pulmonary fibrosis (39,40). The underlying mechanism consists of a complex immunological process, characterized by immune complexes formation, which trigger a neutrophilic recruitment and activation, and delayed hypersensitivity involving a Th1-

cell mediated immune response (12,40). Since only a small proportion of exposed individuals develop the disease and both its presentation form and evolution varies, it has been hypothesized that genetic predisposition plays a role in disease expression.

IFN- γ is one of the key cytokines in T1 (Th1 and Tc1) lymphocyte responses and is classically associated with diseases characterized by granuloma formation (55). Specifically, in HP, T1 lymphocyte responses are deleterious, and it has been proposed that INF- γ is a relevant mediator in granuloma formation and progression to fibrosis. Experimental models have shown that IFN- γ knockout mice developed minimal inflammatory response and no granulomas when exposed to a standardized antigen preparation; furthermore, when IFN- γ was administered, these mice developed granulomatous lung inflammation (56). In a similar model, IFN- γ knockout mice had a decrease in the lung recruitment of

CXCR3⁺/CD4⁺ T cells and impaired granuloma formation, associated with the reduced levels of the IFN- γ -induced chemokines (the CXCR3 ligands: CXCL9, 10 and 11) (57). These CXCR3/CXCL10 interactions were also shown to be involved in HP in humans, namely the CXCL10 expression and secretion by alveolar macrophages in response to IFN- γ , resulting in the lung recruitment of T1 lymphocytes (58,59). Other experimental studies found that treating previously exposed mice with monoclonal antibody anti-IFN- γ associates with a decrease in lung inflammatory and fibrotic response (60) and also that the protective role of CD4⁺/CD25⁺ T regulatory cells in HP is achieved through IFN- γ production suppression (61).

Additionally, and in order to highlight IFN- γ role in HP, Nance and co-workers (62) reported that not only T-cells were able to produce IFN- γ , as innate immune cells are also an important source, and sufficient to granuloma formation. These findings were supported by a study in which IL-4-producing natural killer (NK) T-cells exerted a protective role in experimental HP development by suppressing IFN- γ -producing neutrophils (63).

In our study, although no differences were found in genotype distributions of IFN- γ (+874 T/A) polymorphism between HP patients and exposed controls, the IFN- γ T/T genotype and IFN- γ T allele frequency, which associate with higher IFN- γ expression levels (52), were higher among patients with chronic presentation. In fact, a more indolent clinical presentation might be explained by the higher IFN- γ expression levels, possibly related to an increased and persistent T1-mediated (delayed) inflammation, perpetuating granuloma formation and enabling subsequent evolution to chronicity.

We did not find association of the IL-6 (-174) gene polymorphism and pigeon breeder's disease, as the genotype distributions and allelic frequencies had no differences between HP patients and exposed controls. This was corroborated by data from another study in Japan, also including summer-type HP (34). However, the IL-6 -174C/C genotype, which has been associated with reduced IL-6 expression and serum levels (31), was more frequent among patients who evolved to chronicity, i.e. those with no resolution, one year after the initial diagnosis. On the other hand, studying a limited number of 8 HP cases, Vasakova et al. (25) showed that the IL-6 -174C/G

genotype associated with higher ENA-78 BALF levels, an angiogenic CXC chemokine that may participate in tissue repair (64), suggesting a possible role of IL-6 polymorphisms on HP prognosis. Interestingly, Grutters et al (65), studying 248 sarcoidosis patients from two different European countries (each with their own controls), found the IL6 -174C allele frequency increased in Stage IV sarcoidosis, suggesting it might also have a role in the genetics underlying sarcoidosis severity or progression. Moreover, this polymorphism was also suggested by Jung et al. (66) to be related to vasculitis' susceptibility, especially for large and medium vessels. In a HP mice model, the administration of neutralizing anti-IL-6 antibody was associated with a more robust neutrophilic and an fibrotic response; conversely, when IL-6 was given, the inflammatory cell recruitment and fibrotic response diminished (67). In humans, a case control study also showed that pigeon breeder's disease patients had significantly lower BALF IL-6 levels than their asymptomatic counterparts, supporting a mechanism through IL-6 production of downregulation the lung inflammatory response to antigen exposure (68) with impact in its resolution.

TNF- α is a pleiotropic cytokine, primarily produced by activated macrophages, with broad immune regulation functions that has been closely associated to the development of HP (69). The allele frequency of TNF-2 allele (A at position-308, associated with high TNF- α production) was reported to be higher in farmer's lung disease patients (28). Camarena et al. (35) also showed a probable relevance of TNF-2⁻³⁰⁸ allele in HP susceptibility, as it was more frequently found in PBD patients than in control groups ($p < 0.05$). However, the comparison of TNF- α concentrations, either in BAL or plasma, showed no differences between patients exhibiting TNF-2⁻³⁰⁸ with those with other TNF- α polymorphisms. In our population, no differences were found in these polymorphisms among cases and exposed but healthy subjects. Similarly, Kondoh et al (34), in a study carried out in Japan, did not find any significant difference in TNF- α polymorphisms in HP patients.

IL-10 is an important anti-inflammatory cytokine that can inhibit distinct proinflammatory cytokines production, including TNF- α , IFN- γ and IL-1 β by Th1 cells (70). High- and intermediate-producers IL-10 genotypes were more frequent in sarcoidosis patients compared with healthy controls,

suggesting its role in other granulomatous lung diseases (71). In a murine model of farmer's lung, IL-10 knockout mice had a more severe granulomatous inflammatory response than normal mice (72). Another study demonstrated that patients with HP caused by diisocyanate showed decreased IL-10 mRNA expression levels in antigen-stimulated peripheral blood mononuclear cells, whereas cells from exposed but asymptomatic subjects responded with enhancement in IL-10 mRNA expression (73). TGF- β is another cytokine that is consistently found to be upregulated in various experimental fibrotic diseases (18), and higher TGF- β BAL levels have been reported in HP patients than in normal subjects (74). In our study, we did not find an increased susceptibility to HP related to the studied IL-10 and TGF- β polymorphisms, findings also corroborated by Kondoh et al (34). Also, Yang et al (75) did not find differences in the same IL-10 genotypes and alleles between PBD patients, healthy exposed and unexposed controls.

The present study has a few limitations. Firstly, only a small number of patients were enrolled and the sample size limits the statistical power. Secondly, each cytokine gene polymorphism was analysed independently and, most probably, there are other factors influencing HP phenotype, namely other genetic factors such as HLA or chemokines (76,77), or exposure features such as antigen dose, its dispersion within the respiratory tract or systemic dissemination and the repetitive, chronic nature of the exposure (40). Although the diagnosis was established with a MDT approach, HP diagnostic criteria are still not validated across different centres. Regarding the healthy exposed controls, although they had no clinical or imagiological suspicion of respiratory disease (unrelated healthy bone marrow donors, with domestic avian exposure), they were not followed up or subjected to a more detailed evaluation (lung function, HRCT scan) in order to a definite exclusion of a subclinical HP. The study has also several strengths, such as the prospective design, enrolling consecutive cases in our reference centre with a well-defined exposure and diagnosed by the same DLD multidisciplinary team. Also, patients' follow-up was taken under the care of the same experienced team and long enough to determine the definitive disease evolution at least one year after exposure cessation. Finally, only patients with PBD, which represent a subgroup of HP, were studied. In fact, the relevance

of the antigen type in HP phenotype and prognosis is still controversial. (78–81). Since we only included HP patients with the same type of avian exposure, we avoided the influence of a heterogeneous antigen exposure on our analysis. Our findings supporting the influence of cytokine gene polymorphisms in the different patterns of HP expression, may also suggest that a different genetic background is at least partially responsible for the natural history of progressive fibrosing DLD. In our understanding, different genetic polymorphisms may be clinically relevant for this pattern of disease progression, with the potential to identify patients with more accelerated lung function decline and health-related quality of life deterioration that may have treatment implications (82–85).

In conclusion, in PBD, genotype and allele frequencies of the studied INF- γ , IL-6, TNF- α , TGF- β , and IL-10 polymorphisms were not significantly different from unrelated healthy exposed controls of the same ethnic origin. However, both the IFN- γ T/T (high) and the IL-6 C/C (low) genotypes may play a role in the genetic predisposition underlying a different disease expression, as their frequencies are significantly increased in HP patients due to avian exposure with chronic presentation or no disease resolution one year after the initial diagnosis, respectively. Future collaborative and more extensive genetic studies will be needed to corroborate these findings.

ACKNOWLEDGEMENTS

We acknowledge M. Beltrão, MSc for the contribution on the bronchoalveolar lavage analysis.

N. Martins would like to thank the Portuguese Foundation for Science and Technology (FCT-Portugal) for the Strategic project ref. UID/BIM/04293/2013 and "NORTE2020 - Northern Regional Operational Program" (NORTE-01-0145-FEDER-000012) and the Horizon 2020 Program (PTDC/PSI GER/28076/2017).

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SUPPLEMENTARY TABLE 1. Comparison of cytokine genotypes frequencies between HP patients and controls.

Genotype (expression)	HP patients (N = 40)	Exposed Controls (N = 70)	OR	CI 95%	<i>p</i> value†
TNF-α					
A/A (high)	2 (5%)	4 (5.7%)	0.87	0.15-4.98	1
G/A (high)	7 (17.5%)	22 (31.4%)	0.46	0.18-1.2	0.17
G/G (low)	31 (77.5%)	44 (62.9%)	2.04	0.84-4.95	0.17
TGF-β					
C/C C/C (low)	0 (0%)	1 (1.4%)	0	--	1
C/C G/C (low)	2 (5%)	2 (2.9%)	1.79	0.24-13.22	0.96
C/C G/G (int)	4 (10%)	3 (4.3%)	2.48	0.53-11.69	0.44
T/C G/C (int)	3 (7.5%)	4 (5.7%)	1.34	0.28-6.31	1
T/C G/G (high)	16 (40%)	33 (47.1%)	0.75	0.34-1.65	0.60
T/T G/G (high)	15 (37.5%)	27 (38.6%)	0.96	0.43-2.14	1
IFN- γ, n (%)					
A/A (low)	16 (40%)	26 (37.1%)	1.13	0.51-2.51	0.93
T/A (int)	19 (47.5%)	29 (41.4%)	1.28	0.59-2.8	0.68
T/T (high)	5 (12.5%)	15 (21.4%)	0.52	0.17-1.56	0.36
IL-6, n (%)					
C/C (low)	5 (12.5%)	9 (12.9%)	0.97	0.3-3.12	1
G/C (high)	15 (37.5%)	32 (45.7%)	0.71	0.32-1.57	0.52
G/G (high)	20 (50%)	29 (41.4%)	1.41	0.65-3.08	0.50
IL-10, n (%)					
ACC/ACC (low)	5 (12.5%)	9 (12.9%)	0.97	0.3-3.12	1
ACC/ATA (low)	5 (12.5%)	10 (14.3%)	0.86	0.27-2.72	1
ATA/ATA (low)	3 (7.5%)	6 (8.6%)	0.86	0.2-3.64	1
GCC/ACC (int)	10 (25%)	21 (30%)	0.78	0.32-1.88	0.73
GCC/ATA (int)	10 (25%)	18 (25.7%)	0.96	0.39-2.35	1
GCC/GCC (high)	7 (17.5%)	6 (8.6%)	2.26	0.7-7.27	0.28

HP Hypersensitivity pneumonitis; OR Odds Ratio; CI Confidence Interval; †Chi-square test.

SUPPLEMENTARY TABLE 2. Comparison of cytokine alleles frequencies between HP patients (n=40) and controls (n=70).

Allele	HP patients	Exposed Controls	OR	CI 95%	<i>p</i> value†
TNF-α -308					
A	11 (13.8%)	30 (21.4%)	0.58	0.28-1.24	0.159
G	69 (86.2%)	110 (78.6%)	1.71	0.81-3.63	
TGF-β codon10					
C	31 (38.8%)	49 (35%)	1.17	0.67-2.07	0.578
T	49 (61.2%)	91 (65%)	0.85	0.48-1.5	
TGF-β codon25					
C	5 (6.2%)	8 (5.7%)	1.1	0.35-3.48	0.871
G	75 (93.8%)	132 (94.3%)	0.91	0.29-2.88	
IFN-γ +874					
A	51 (63.8%)	81 (57.9%)	1.28	0.73-2.26	0.391
T	29 (36.2%)	59 (42.1%)	0.78	0.44-1.37	
IL-6 -174					
C	25 (31.2%)	50 (35.7%)	0.82	0.46-1.47	0.502
G	55 (68.8%)	90 (64.3%)	1.22	0.68-2.2	
IL-10 -1082					
A	46 (57.5%)	89 (63.6%)	0.78	0.44-1.36	0.374
G	34 (42.5%)	51 (36.4%)	1.29	0.74-2.26	
IL-10 -819					
C	59 (73.8%)	100 (71.4%)	1.12	0.61-2.09	0.711
T	21 (26.2%)	40 (28.6%)	0.89	0.48-1.65	
IL-10 -592					
A	21 (26.2%)	40 (28.6%)	0.89	0.48-1.65	0.711
C	59 (73.8%)	100 (71.4%)	1.12	0.61-2.09	

HP Hypersensitivity pneumonitis; OR Odds Ratio; CI Confidence Interval; †Chi-square test.