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Original articles

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A major genetic determinant of autoimmune diseases is associated with the presence of autoantibodies in Hypersensitivity Pneumonitis

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Abstract

Background: Hypersensitivity pneumonitis (HP) is an immune-mediated disease triggered by exposure to organic particles in susceptible individuals. It has been reported that a subgroup of patients with HP develops autoantibodies with or without clinical manifestations of autoimmune disease. However, the mechanisms involved in this process and the effect of the autoantibodies on clinical course in HP is unknown. We evaluated the association between HLA class II alleles and HP patients with and without autoantibodies.

Methods: One hundred seventy HP patients were included. We analyzed the presence of antinuclear antibodies, rheumatoid factor, anti-SSA/Ro, anti-SSB/La, and anti-CCP at the time of diagnosis. In addition, in a subset of patients, we evaluated anti-Scl-70, ANCA, and anti-DNA. HLA typing was performed by PCR-SSP in a high-resolution modality, including *HLA-DRB1* and *HLA-DQB1* loci. Statistical analysis was performed employing Epi-Info v7 and SPSSv20.

Results: Sixty HP patients showed sera autoantibodies (HPAbs+), and 110 HP patients did not (HPAbs-). The frequency of the allele *HLA-DRB1*03:01* was remarkably increased in the HPAbs+ group (10.8% *vs*. 0.45%; OR=30.14, 95%CI 3.83-237.1; p=1.65E-04 after Bonferroni's correction). Likewise, we found that the haplotype *DRB1*03:01-DQB1*02:01,* which is part of the 8.1 ancestral haplotype, a major genetic determinant of autoimmune diseases confers significant risk to develop autoantibodies (OR=19.23, 95%CI 2.37-155.9; p=0.0088 after Bonferroni's correction). Also, the *HLA-DRB1*03:01* allele was associated with higher mortality in patients with HP (adjusted OR=5.9, 95%IC 1.05-33.05; p=0.043).

Conclusions: A subset of HP patients presents circulating autoantibodies and higher mortality, that are associated with some alleles of 8.1 ancestral haplotype.

Keywords: Hypersensitivity pneumonitis; Genetic susceptibility; HLA polymorphisms; Autoantibodies.

Introduction

Hypersensitivity pneumonitis (HP) is an interstitial lung disease (ILD) caused by an exaggerated immune response to the inhalation of a wide variety of organic particles in genetically susceptible individuals. [1–5] The prognosis of acute HP is usually favorable; however, chronic fibrotic HP often progresses, with the subsequent destruction of the lung architecture. [6,7]

The heterogeneous nature of the immune response and the clinical differences through the disease suggest that multiples molecular pathways are involved in the development and progression of the disease.

Previous studies have described the participation of some alleles from the major histocompatibility complex (MHC) in HP susceptibility. Particularly, HLA class II alleles were identified in patients with pigeon breeder's disease and summer-type HP [8–10] suggesting that genetic factors located inside the HLA region contribute to HP development. On the other hand, and relevant for this study, class II -*DRB1* and -*DQB1* alleles involved in 8.1 ancestral haplotype (AH) seem to determine the development of specific autoantibodies, both organ and non-organ specific. [11]

In 2016 a cohort of patients with chronic HP was examined by Adegunsoye et al. to determine the prevalence of autoimmune characteristics. They found that a significant minority of patients with HP (15%) displayed autoimmune features and that the presence of autoimmunity was an independent predictor of the increased mortality. [12]

Throughout the patients' evaluation with HP, we have often detected the presence of circulating autoantibodies but without fulfilling the criteria for a connective tissue disease. To date, it is unknown whether this subgroup of patients has a different genetic susceptibility associated with HLA compared to the HP autoantibodies negative group.

In this context, this study aimed to examine the allele frequencies of class II HLA alleles to reveal putative differences between HP with and without autoantibodies, and whether some polymorphisms are associated with mortality rate.

Material and methods

Study population

A retrospective review of adult patients (≥ 18 years) referred to the ILD clinic at the National Institute of Respiratory Diseases in Mexico City was conducted, and samples with enough DNA quality from 170 patients with a confirmed diagnosis of HP between the period 2003 to 2018 were included (**Figure 1**). DNA samples for genetic studies are obtained in patients with interstitial lung diseases (ILD) from the year 2000, with clear explanation and signed informed consent is duly collected.

All patients had at least prior three generations born in Mexico (parents and grandparents) and were considered as Mexican mestizo. We have previously demonstrated that this criterion is a good proxy of Mexican ancestry evaluated by ancestry-informative markers. [13]

HP was diagnosed according to the previously described criteria. [14,15] [1] Symptoms and pulmonary function tests alterations compatible with interstitial lung disease. [2] High-resolution computed tomography showing poorly defined nodules, ground-glass attenuation and air trapping in expiration; (80% of the HPAbs+ and 54% of the HPAbs-) also showed alterations compatible with fibrosis such as irregular linear opacities and traction bronchiectasis and occasionally cystic lesions; [3] BAL lymphocytosis >30% [4] biopsy (performed in 30% of the HPAbs+ and 28% of the HPAbs-) compatible with HP. A multidisciplinary team confirmed the diagnosis.

Clinical data were obtained from the medical records. The variables collected included demographical data, comorbidities, tobacco smoking history, environmental antigen exposure assessment, clinical laboratory studies, pulmonary function tests, including forced

vital capacity (FVC), diffusion capacity of the lung for carbon monoxide (DL_{CO}) and six-minute walk test, and mortality from all causes at one year follow up.

We considered as HP with positive serology when at least one of the following antibodies where positive: antinuclear antibodies (ANAs) with a specific pattern of connective tissue disease of any kind (cytoplasmic, nucleolar, centromere); ANA with homogeneous pattern, fine or coarse mottle; rheumatoid factor ≥3 times the upper normal limit (20 IU/ml); anti-cyclic citrullinated peptide (anti-CCP) ≥20 and/or at least one antibody in the autoimmunity profiles for Sjogren syndrome [(anti-Ro (SSA), anti-La (SSB)]. These autoantibodies were analyzed in all the patients. Also, autoantibodies associated with systemic sclerosis (anti-Scl-70), vasculitis (anti-neutrophil cytoplasmic antibody, ANCA), and anti-DNA were examined in 88% of the HPAbs+ and 55% of the HPAbs-. No patient met classification criteria for connective tissue diseases according to the American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) [16–18].

The patients were classified according to the presence of autoantibodies present in serum (HPAbs+) *versus* those seronegative (HPAbs-) (Table 1).

Ethics statement

This study was approved by the Institutional Committee for Science and Ethics of the Instituto Nacional de Enfermedades Respiratorias Ismael Cosío Villegas (INER) (approbation codes: B20-15 and C60-17).

Genomic DNA isolation

Peripheral blood (7 mL) was obtained by venipuncture and was collected in a tube with EDTA as an anticoagulant for subsequent DNA extraction. Samples were obtained at the time of diagnosis, and patients were not receiving corticosteroids or immunosuppressive drugs.

The blood samples were handled in the HLA laboratory of INER to obtain genomic DNA employing a commercial kit (BDtract Genomic DNA Isolation Kit, Maxim Biotech, Inc. San Francisco, CA, USA). The DNA was quantified by UV spectrophotometry using a NanoDrop 2000 device (Thermo Scientific, DE, USA). Contamination with organic compounds and proteins was defined by measuring the ratio absorbance at 280 nm and 260 nm. Samples were considered of good quality when the ratio was ~1.8.

HLA class II molecular typing

Molecular typing for DRB loci and *DQB1* locus was done by PCR sequence-specific primers (PCR-SSP) technique using the Micro SSP™ HLA DNA Typing Trays (One Lambda, Inc., Canoga Park, CA, USA). Unlike other PCR-based methods, the SSP methodology employed discriminates between the different alleles during the PCR process. The amplified DNA fragments are separated by agarose gel electrophoresis and visualized by staining and exposure to ultraviolet light. The interpretation of PCR-SSP results is based on the presence or absence of a specific amplified DNA fragment. Since amplification during the PCR reaction may be adversely affected by various factors such as pipetting errors, poor DNA quality, and presence of inhibitors, an internal control primer pair was included in every PCR reaction. The control primer pair amplifies a conserved region of the Human β-globin gene, which is present in all human DNA samples and is used to verify the integrity of the PCR reaction.

Initially, HLA-DRB typing was performed by low-resolution modality, which includes *DRB1*, *DRB3*, *DRB4*, and *DRB5* alleles in 24 independent reactions. DRB loci allele group covers *DRB1*01*, *03, *04, *07, *08, *11, *12, *13, *14, *15 and *16 as well as *DRB3*02*, *DRB3*03*, *DRB4*01*, *DRB5*01* specificities (DR51, DR52 and DR53 serological equivalents). High resolution for *HLA-DRB1*03* was performed with an additional panel of 16 primer pairs to establish allelic discrimination. For *HLA-DQB1* typing, eight primer pairs were used, while

the *HLA-DQB1* high-resolution kit used to achieve *DQB1* amplification, which includes 55 alleles for DQ6, DQ3, DQ4, DQ5, and DQ2.

All amplifications were performed using the Kapa Taq HotStart PCR kit (Kapa Biosystems. Cape Town, South Africa). PCR-SSP products were electrophoresed in 3% agarose gels stained using MIDORI Green Advanced DNA Stain (Nippon Genetics Europe, Dueren, Germany). Interpretation of typing results was made using the assistance of the HLA Fusion Software v. 4.3 (One Lambda, Inc. Canoga Park, CA. USA).

Statistical analysis

The statistics program SPSS v.21 (SPSS Inc., Chicago, IL, USA) was used to describe the study population and determine the median, minimum, and maximum values for each variable and compared using Mann-Whitney U Test. Continuous variables were reported as means with standard deviation (SD) and analyzed using a Student's t-test. Categorical variables were reported as counts and percentages and contrasted using Fisher's exact test.

Allele and haplotype frequencies of HLA determined by direct counting. The observed and expected HLA class II alleles (each locus) tested for Hardy-Weinberg equilibrium using a conventional Fisher's exact test. The associations were evaluated by Fisher's exact two-tailed test, with a statistical significance value of p (<0.05). Bonferroni correction was carried out considering the twenty alleles identified in both loci and the 22 haplotypes found in the whole population. Odds ratios (OR) and 95% confidence intervals were calculated using 2x2 contingency tables comparing both groups by alleles and haplotypes reported using the Epi Info v4.0.1 [19]. Finally, the haplotypes were constructed employing Arlequin v3.1 software [20] using the Maximum-likelihood method, with an iterative EM algorithm.

To evaluate the strength of association between mortality and the risk allele, we first estimated a crude OR (cOR) using univariate regression analysis; the p-value was estimated with the Wald test, and then we estimated an adjusted OR (aOR) with a multivariate logistic

regression analysis that includes only variables with a p-value <0.05, due to the small sample size. No imputations procedures were performed to handle missing data.

Results

Of 298 patients registered in the HP cohort, 93 did not have enough inclusion data for the study, including lack of autoantibodies results (before 2006, ILD patients did not undergo the measurement of autoantibodies routinely), or because our multidisciplinary team did not confirm the diagnosis of HP. The other 35 were excluded due to not having enough genomic DNA to perform genotyping. One hundred seventy patients with HP were included, 60 with circulating autoantibodies (HPAbs+) and 110 without (HPAbs-) (**Figure 1**). Most patients HPAbs+ (89%) were positive for antinuclear antibodies (>1:320), and occasionally, they were also positive for another of the autoantibodies examined (see methods). Twelve percent (7 patients) were negative for ANA but showed increased levels of other autoantibodies (e.g., RF, two patients, ANCA 2 patients, anti-Ro two patients, anti-La one patient). HPAbs- were negative for all examined autoantibodies.

Demographic characteristics

There were no differences between HPAbs+ and HPAbs- regarding demographic characteristics, including age, gender, and smoking. (**Table 1**). Likewise, the comparison of pulmonary function tests between both groups (performed in 49 of 60 HPAbs+ and 85 of 110 HPAbs- patients) showed no differences (**Table 1**). However, patients HPAbs+ were more likely to have chronic/fibrotic changes in the HRCT that HPAbs-. In contrast, the percent of patients in which we were unable to identify the antigen was significantly higher in the group of HPAbs- (**Table 1**).

During the one-year follow-up, six patients HPAbs+ and two patients from the HPAbs- were lost. Twelve percent of the entire cohort died, with a significantly higher proportion in the HPAbs+ group (20% *vs.* 8%, p = 0.02).

HLA class II alleles and haplotypes

No statistically significant differences were found in the population stratification based on the region of origin between cases and controls. The HLA allele pairs of each locus from both groups satisfied Hardy-Weinberg Equilibrium (p>0.05), accounting for common alleles producing common genotypes, i.e., both heterozygous (e.g., *DQB1*02:01*, *DQB1*05:01*) and homozygous (e.g., *DQB1*03:02*) pairs.

The distributions of *DRB1* and *DQB1* alleles are listed in **Tables 2 and 3**. Ten *HLA-DRB1* alleles were identified in the HPAbs+ group, seven of them with an allelic frequency (FA) greater than 5%, and the most common were *DRB1*08* (AF = 20.00) and *DRB1*04* (AF = 17.50). A marked increase in the frequency of *HLA-DRB1*03* was identified in the group HPAbs+ (10.8% versus 0.45%; OR = 30.14, 95% CI 3.83 - 237.1; p=1.65E-04 after Bonferroni's correction for the total of alleles, (20 identified in both loci). All *DRB1*03* subjects were *DRB1*03:01* in the high-resolution subtyping analysis. The alleles in the *DRB3* and *DRB4* genes were found neither in HPAbs+ nor in HPAbs-, while *DRB5* was showed in few patients without differences.

Seven alleles were recognized at the *HLA-DQB1* locus, among them *DQB1*03:01*, *DQB1*03:02* and *DQB1*04:02* were the most frequent in both groups. A higher frequency of the allele *DQB1*02:01* (AF = 15.00) was detected in the HPAbs+ group compared with HPAbs- (15.0% vs. 6.364%, p = 0.007, OR = 2.93, 95% CI 1.33 - 6.45); however, this association was lost after Bonferroni's correction (p = 0.14). The frequency of the *HLA-DQB1*06:01* allele was also more than twice-fold higher in the HPAbs+ group (12.5% vs. 4.5%, p = 0.006, OR = 3.33, 95% CI 1.39 - 7.98), but also, the Bonferroni correction revealed that this difference was not significant (p = 0.12).

We also compared the haplotype frequency (HF) of the *DRB1-DQB1* loci. As shown in **Table 4**, twenty-two haplotypes were identified in the whole HP group; interestingly, in the HPAbs+

group (n = 60) are nineteen haplotypes, while in the HPAbs- (n = 110) only fourteen haplotypes were determined (19/22 vs. 14/22, p = 0.08).

We observed a significant higher frequency of the haplotype *DRB1*03:01-DQB1*02:01* in patients with HPAbs+ (7.5% versus 0.45%; OR = 19.23, 95% CI 2.37 - 155.9; p = 0.0088 after Bonferroni's correction (applying the 22 haplotypes identified in the whole population).

Logistic regression

To evaluate the strength of the association between mortality and the risk allele, we estimated the crude OR (cOR) using univariate logistic regression analysis of mortality. The risk allele *HLA-DRB1*03:01* was associated to mortality (cOR = 4.5, 95% CI 1.19-16.9; p = 0.026). To adjust for confounding, we performed three multivariate logistic regression models (**Table 5**). In model one (M1), we included the four variables that showed significant association with mortality by univariate analysis: *HLA-DRB1*03:01*, DLCO, CRP, and HPAbs+. We found that DLCO and CRP remained significant. In model 2 (M2) when we excluded HPAbs+, the allele *HLA-DRB1*03:01* was strongly associated with mortality (aOR = 5.9, 95% CI 1.05 – 33.0; p = 0.043). By contrast, when we excluded the allele *HLA-DRB1*03:01* (M3), HPAbs+ lost significance, supporting the strong association with the risk allele. Importantly, *HLA-DRB1*03:01* and HPAbs+ were strongly associated [OR= 9.52 (95% CI 1.85-92)], indicating collinearity between these two variables.

The multivariate logistic regression model was obtained with the data of 103 patients, the goodness of fit of the M2 is p = 0.69, and the area under ROC curve = 0.82 (**Table 5**).

Discussion

Studies about HP genetic susceptibility are limited, but HLA class II genes have been identified as critical factors that contribute to disease development [9,10,21]

In the last few years, a growing body of evidence indicates that a subgroup of patients with HP develops serum or even clinical features of autoimmunity, although the underlying mechanisms are still unknown. Moreover, except for higher mortality, this “phenotype” has not been characterized.

Similarly, in the last years, it has been described a subset of patients with idiopathic interstitial pneumonia (IIP) and autoimmune features (IPAF) who did not meet the criteria for a connective tissue disease (CTD) but have at least one sign or symptom suggestive of a CTD and at least one serologic test reflective of an autoimmune process. [22] These patients have been better characterized, and some studies also suggest that patients who met the IPAF criteria had a significantly worse survival than those with IIP without autoimmune features [23], although substantial variability in outcome has been reported. [24] As in HP, the mechanisms and genetic susceptibility triggering the autoimmune process is unknown.

In this study, we found that around a third of the patients with HP may present autoantibodies. This proportion is higher than reported by Adegunsoye et al. [12], the only study that to our knowledge have shown this association, although the reasons are currently unknown.

In this study, we aimed to identify putative genetic factors associated with the HLA class II system that may increase the risk of developing autoimmune features in patients with HP. Our results revealed, by the first time, some of the genetic alleles conferring risk to develop autoantibodies in these patients. Thus, a significant increase in the frequency of the *HLA-DRB1*03:01* allele as well as the haplotype *DRB1*03:01-DQB1*02:01* was observed in the group of patients with autoantibodies compared with patients without them.

Interestingly, these alleles have been described in the formation of autoantibodies in classic and rare autoimmune disorders. For example, alleles *HLA-DRB1*03:01* and *DQB1*06:01* are associated with the development of autoantibodies in systemic lupus erythematosus [25,26]. Likewise, the presence of anti-Jo-1 or anti-PM-Scl antibodies was found strongly associated with *HLA-DRB1*03:01* and *DQA1*05:01* alleles in a cohort of patients with adult and juvenile myositis [27–29]. The *DQB1*02:01* allele is linked genetically to *DQA1*05:01* and is classically described in celiac disease, type 1 diabetes, and other autoimmune disorders [30].

Haplotype analysis revealed a significant increase in the frequency of *HLA-DRB1*03:01-DQB1*02:01*, commonly observed in the European population [31] and associated with several autoimmune disorders, as primary Sjogren syndrome with autoantibodies production. [32] This haplotype contains two main alleles that are part of 8.1 ancestral haplotype (AH). The AH is exceptionally long (>4 megabases) conserved combination of alleles: *HLA-A1*, *Cw7*, *B8*, *TNFAB*a2b3*, TNFN*S, *C2*C*, Bf*s, *C4A*Q0*, *C4B*1*, *DRB1*03:01*, *DRB3*01:01*, *DQA1*05:01*, *DQB1*02:01*. It is accepted as the most critical immunologic determinant conferring risk to several autoimmune diseases. [11] Moreover, even healthy carriers of this haplotype show high values of autoantibodies, blood-activated T-cells, and blood immune complexes. Therefore, 8.1 AH enhance immune dysfunctions and autoimmune disorders, and our results suggest that it may also contribute to the development of autoimmunity in HP.

HP is a complex disease, where the interaction of environmental and genetic factors influences the development of the disease and the phenotype, e.g., fibrotic or non-fibrotic disease. [4] For example, it has been recently demonstrated that the minor allele frequency of *MUC5B* rs35705950 is higher in patients with chronic HP than in healthy controls, and more importantly, that the extent of radiographic fibrosis is associated with this common variant. [33]

Our findings suggest that the presence of specific alleles and haplotypes of the HLA system may lead to the development of autoimmune features, modifying the course and outcome of

HP. Moreover, to carry the risk allele, *HLA-DRB1*03:01* was associated with reduced survival. Interestingly, immune dysfunctions associated with 8.1 AH, appear to be a factor to early morbidity and mortality in older women,[34] gender that was markedly more frequent in our HP cohort.

We also found that mortality in the group of HPAbs+ was also higher than in the group without autoantibodies, coinciding with a previous study in which the presence of autoimmunity was found associated with poor prognosis [12]. However, after adjusting by the allele *HLA-DRB1*03:01*, the presence of autoantibodies lost significance. Surprisingly, mortality did not show differences between chronic fibrotic and non-fibrotic HP patients, likely because of the short time of follow-up and the small sample size.

Our research has some limitations, including the relatively small sample size. Besides, we excluded patients with HP and confirmed connective tissue disease or a history of inflammatory disorders.

Further studies are needed to validate these findings and to determine the molecular mechanisms of the underlying autoimmune process in patients with hypersensitivity pneumonitis.

Conclusion

In patients with hypersensitivity pneumonitis, the allele *HLA-DRB1*03:01* and the haplotype *DRB1*03:01-DQB1*02:01*, which is part of the 8.1 ancestral haplotype, a major genetic determinant of autoimmune diseases, are associated with the presence of autoantibodies. HLA-DRB1*03:01 also increases the risk of mortality in patients with HP.

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Table 1

Clinical and demographic characteristics in HP subgroups

Characteristics	n	HPAbs+	n	HPAbs-	p-value
Age, years ± SD	60	53 ± 9	110	53 ± 12	0.59
Gender, female (%)	60	52 (86)	110	89 (81)	0.39
Duration of symptoms before diagnosis, months.	60	25 (4 - 20)	110	27 (2 - 120)	0.30
Former smoker, (%)	60	14 (23)	110	26 (24)	0.96
Exposure to birds, (%)	60	47 (78)	110	91 (83)	0.53
Diabetes mellitus, (%)	60	11 (18)	110	16 (15)	0.51
Systemic hypertension, (%)	60	9 (15)	110	22 (20)	0.53
Dead*, (%)	54	12 (20)	108	9 (8)	0.02
Antibodies against avian antigens (%)	60	59 (94)	110	57 (53)	<0.0001
Unidentified antigen	60	4 (6)	110	50 (47)	<0.0001
HRCT chronic pattern (%)	60	48 (80)	110	59 (54)	<0.001
C-reactive protein, mg/dl	60	0.9 (0.02 – 7.1)	110	0.7 (0.01 – 3.9)	0.95
BAL** lymphocytosis, (%)	49	49 ± 19	87	46 ± 25	0.17
FVC, % predicted	60	57.8 ± 18	106	53.8 ± 20	0.20
DLCO, % predicted	49	46 ± 22	85	51 ± 21	0.24
6MWT, meters	42	333 ± 168	71	333 ± 116	0.21
pO2***, mm Hg	42	51 ± 8	71	50 ± 13	0.90
PAP****, mm Hg	47	39 ± 14	69	38 ± 14	0.40

Mean ± SD; median (minimum and maximum values). *Data for deaths were accounting in 54 HPAbs and 108 HPabs- patients. ** BAL was performed in 81% of the HPAbs+ and 79% of the HPAbs-, ***pO2 oxygen pressure was measured in 42 HPAbs+ and 71 of the HPAbs -; ****PAP: pulmonary artery pressure (measured by echocardiography in 47 HPAbs+ and 69 of the HPAbs-). HRCT: high resolution computed tomography; HP: hypersensitivity pneumonitis; Abs: antibodies; 6MWT: 6 min walk test.

Table 2

Frequencies of HLA-DRB1 alleles in patients with HP with and without autoantibodies

DRB1	HP (all, n=170)		HPAbs+ (n=60)		HPAbs- (n=110)		p-value	OR	95% CI
	n	AF (%)	n	AF (%)	n	AF (%)			
*01	17	5.000	5	4.167	12	5.455	0.79	0.74	0.24 - 2.21
*03:01	14	4.118	13	10.833	1	0.455	1.65E-04*	30.14	3.83 - 237.1
*04	70	20.588	21	17.500	49	22.273	0.25	0.67	0.34 -1.28
*07	22	6.471	9	7.500	13	5.909	0.63	1.31	0.52 - 3.28
*08	71	20.882	24	20.000	47	21.364	0.74	0.89	0.47 - 1.69
*09	4	1.176	ND	-	4	1.818	-	-	-
*10	5	1.471	ND	-	5	2.273	-	-	-
*11	24	7.059	6	5.000	18	8.182	0.35	0.56	0.21 - 1.51
*12	2	0.588	ND	-	2	0.909	-	-	-
*13	23	6.765	10	8.333	13	5.909	0.48	1.49	0.61 - 3.64
*14	59	17.353	18	15.000	41	18.636	0.4	0.72	0.36 - 1.41
*15	14	4.118	8	6.667	6	2.727	0.08	2.66	0.87 - 8.08
*16	15	4.412	6	5.000	9	4.091	0.77	1.24	0.42 - 3.68

HP: Hypersensitivity pneumonitis; Abs: Autoantibodies; AF: Allele frequency; OR: Odds ratio; CI: Confidence interval; ND: Not detected. *p-value after Bonferroni's correction for the total of alleles (20) identified in both loci. The comparison was made between HPAbs+ *vs*. HPAbs- groups.

Table 3

Frequencies of HLA-DQB1 alleles in patients with HP with and without autoantibodies

DQB1	HP (all, n=170)		HPAbs+ (n=60)		HPAbs- (n=110)		p-value	OR	95% CI
	N	AF (%)	n	AF (%)	N	AF (%)			
02:01	32	9.412	18	15.000	14	6.364	0.14*	2.93	1.33 - 6.45
03:01	115	33.824	36	30.000	79	35.909	0.12	0.58	0.30 - 1.14
03:02	68	20.000	19	15.833	49	22.273	0.14	0.57	0.29 - 1.11
03:03	4	1.176	ND	-	4	1.818	-	-	-
04:02	72	21.176	25	20.833	47	21.364	1	0.95	0.50 - 1.81
05:01	24	7.059	7	5.833	17	7.727	0.64	0.72	0.28 - 1.85
06:01	25	7.353	15	12.500	10	4.545	0.12*	3.33	1.39 - 7.98

HP: Hypersensitivity pneumonitis; Abs: Autoantibodies; AF: Allele frequency; OR: Odds ratio; CI: Confidence interval; ND: Not detected. *p-value after Bonferroni's correction for the total of alleles (20) identified in both loci. The comparison was made between HPAbs+ *vs*. HPAbs- groups.

Table 4

HLA class II haplotypes in patients with HP with and without autoantibodies

Haplotype	HP (all, n= 170)		HPAbs+ (n= 60)		HPAbs- (n= 110)		p-value	OR	CI 95%
DRB1-DQB1**	n	HF (%)	n	HF (%)	N	HF (%)			
01-03:01	1	0.294	1	0.833	ND	-	-	-	-
01-05:01	16	4.706	4	3.333	12	5.455	0.42	0.58	0.17 - 1.89
03:01-02:01	10	2.941	9	7.500	1	0.455	0.0088*	19.23	2.37 - 155.9
03:01-03:01	1	0.294	1	0.833	ND	-	-	-	-
03:01-06:01	3	0.882	3	2.500	ND	-	-	-	-
04-03:01	1	0.294	1	0.833	ND	-	-	-	-
04-03:02	68	20.000	19	15.833	49	22.273	0.14	0.57	0.29 - 1.11
04-04:02	1	0.294	1	0.833	ND	0.000	-	-	-
07-02:01	22	6.471	9	7.500	13	5.909	0.63	1.31	0.52 - 3.28
08-04:02	71	20.882	24	20.000	47	21.364	0.74	0.89	0.47 - 1.69
09-03:03	4	1.176	ND	-	4	1.818	-	-	-
10-05:01	5	1.471	ND	-	5	2.273	-	-	-

11-03:01	16	4.706	2	1.667	14	6.364	0.05	0.23	0.05 - 1.07
11-06:01	8	2.353	4	3.333	4	1.818	0.45	1.89	0.45 - 7.85
12-03:01	2	0.588	ND	-	2	0.909	-	-	-
13-03:01	18	5.294	5	4.167	13	5.909	0.6	0.67	0.22 - 2.0
13-05:01	3	0.882	3	2.500	ND	-	-	-	-
13-06:01	2	0.588	2	1.667	ND	-	-	-	-
14-03:01	59	17.353	18	15.000	41	18.636	0.4	0.72	0.36 - 1.41
15-03:01	2	0.588	2	1.667	ND	-	-	-	-
15-06:01	12	3.529	6	5.000	6	2.727	0.34	1.92	0.59 - 6.25
16-03:01	15	4.412	6	5.000	9	4.091	0.77	1.24	0.42 - 3.68

HP: Hypersensitivity pneumonitis; Abs: Autoantibodies; HF: Haplotype frequency; OR: Odds ratio; CI: Confidence interval; ND: Not detected.

*p-value after Bonferroni correction for the total of haplotypes (22) identified. The comparison was made between HPAbs+ *vs*. HPAbs- groups.

Table 5

Univariate analysis adjusting the risk allele (DRB1*03:01) by DLCO and C-reactive protein.

Variable	Non-survivors (n=21)	Survivors (n=141)	cOR (95% CI) p-value	aOR (95% CI) [M1] p-value	aOR (95% CI) [M2] p-value	aOR (95% CI) [M3] p-value
Age at baseline evaluation (years)	52.8 ± 9.9	52.6 ± 11.3	1.0 (0.96-1.04) 0.94			
Sex. Male (%)	6 (29%)	22 (16%)	2.1 (0.75 –6.10) 0.15			
DRB1*03:01 (%)	4 (19%)	7 (5%)	4.5 (1.19 -16.90) 0.026	3.9 (0.69 - 24.83) 0.14	5.9 (1.05 – 33.05) 0.043	
Smoking history (current, %)	4 (19%)	33 (24%)	0.7 (0.23–2.40) 0.62			
Arterial hypertension (%)	3 (14%)	26 (20%)	0.6 (0.18–2.40) 0.54			
Diabetes mellitus (%)	3 (14%)	20 (15%)	0.9 (0.25–3.40) 0.91			
Baseline FVC predicted (%)	48 (32–66)	53 (41–73)	0.9 (0.96–1.01) 0.27			
Baseline DLCO predicted (%)	26.5 (20–47)	48.5 (37–64)	0.9 (0.92-0.98) 0.006	0.94 (0.91-0.98) 0.008	0.94 (0.91 – 0.98) 0.007	0.94 (0.91 – 0.98) 0.006
pO2, mmHg	50.9 ± 13.1	50.8 ± 11.0	1.0 (0.95–1.04) 0.99			
pCO2, mmHg	33.7 (30.8–36.0)	35 (32.0–39.9)	0.9 (0.85–1.02) 0.13			
PAP, mmHG	42 (31–50)	35 (25–45)	1.0 (0.99–1.06) 0.09			
6MWT, meters	333 (220-400)	350 (245–434)	0.9 (0.98-1.00) 0.48			
C-reactive protein, mg/dl	0.52 (0.37-1.16)	0.43 (0.23-0.84)	1.6 (1.02-2.55) 0.037	2.09 (1.13-3.86) 0.02	2.2 (1.2 – 3.9) 0.01	2.03 (1.12 – 3.6) 0.02
BAL lymphocytosis, %	40 (33–54)	47 (30-62)	0.9 (0.96-1.01) 0.36			
HRCT chronic pattern (%)	13 (62)	91 (61)	1.03 (0.4-2.65) 0.94			
HPAbs+ (%)	12 (57)	42 (30)	3.14 (1.23 -8.01) 0.02	2.9 (0.83 -10.19) 0.09		3.08 (0.88- 10.7) 0.076

*Survival was obtained in 162 from the 170 patients. cOR: crude OR; aOR: adjusted OR; NS: not significative. 6MWT: 6 min walking test; pO2 oxygen pressure; PAP: pulmonary artery pressure (measured by echocardiography). [M1] Model 1 for adjusted analysis. [M2] Model 2 for adjusted analysis. [M3] Model 3 for adjusted analysis. The cOR of the strength of association DLCO with mortality was estimated with the data of 118 patients.

The multivariate logistic regression model was obtained with the data of 103 patients, the goodness of fit of the model is p = 0.69, and the area under ROC curve = 0.82.

HLA DRB1*03 and HP Abs+ were strongly associated (OR= 9.52 (95% CI= 1.85 – 92.0), suggesting collinearity between these two variables in model 1.

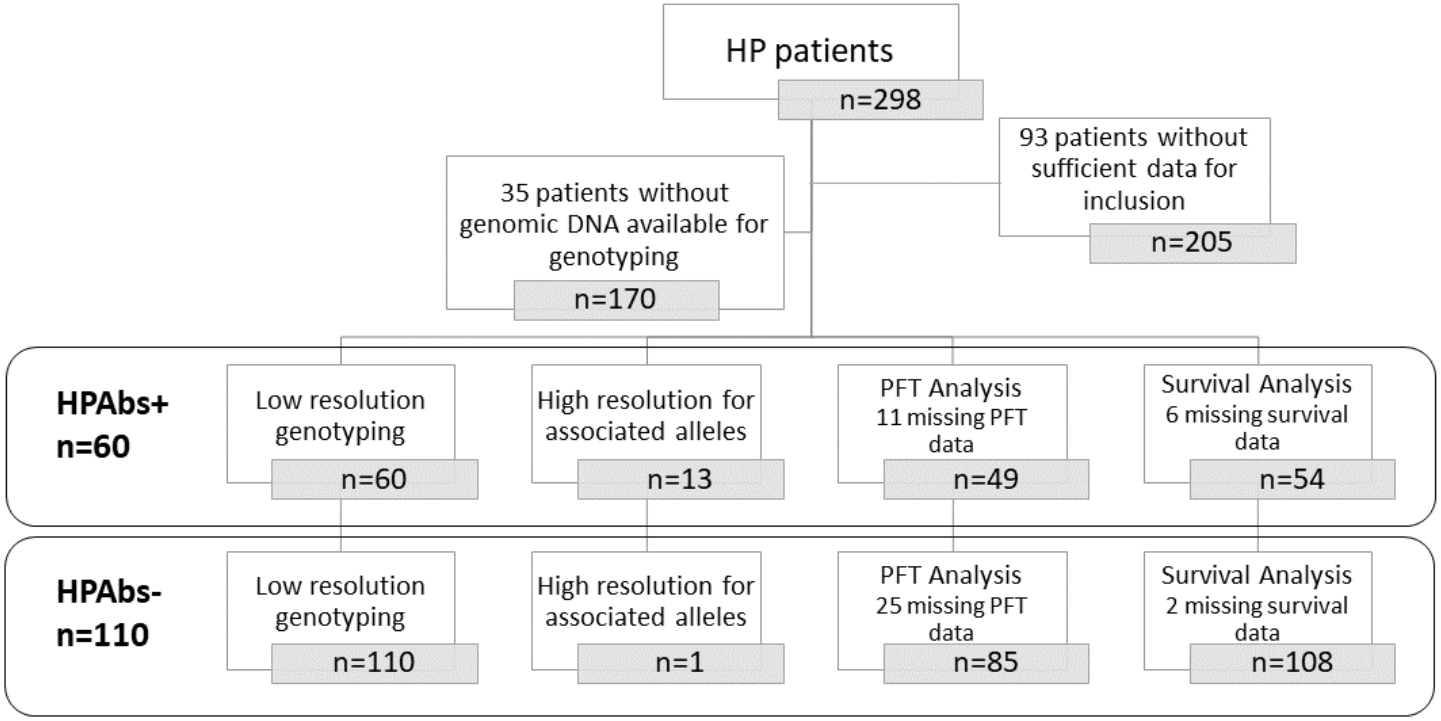

Figure 1. Inclusion of patients.

Exclusion rates for the different analyses. HP: Hypersensitivity pneumonitis; Abs: Autoantibodies; PFT: pulmonary function test.