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The Occurrence of Hermansky Pudlak Syndrome in Patients with Idiopathic Pulmonary Fibrosis—A Cohort Study

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Abstract

Hermansky-Pudlak syndrome (HPS) is an autosomal recessive multisystem disease characterized by bleeding disorders and oculocutaneous albinism. In some patients the syndrome is accompanied by granulomatous colitis, immunodeficiency or pulmonary fibrosis. To date nine genes are associated with the syndrome, resulting in subtypes HPS-1 to HPS-9. Pulmonary fibrosis is most commonly associated with HPS-1. The clinical course of patients with HPS and pulmonary fibrosis resembles that of idiopathic pulmonary fibrosis (IPF). Our aim was to establish the prevalence of HPS in a cohort of IPF patients.

Methods: 127 patients diagnosed with IPF based on the criteria set by the ATS/ERS/JRS/ALAT were retrospectively screened for clinical features of HPS. Genetic analysis was performed in patients with pulmonary fibrosis and 2 or more clinical features of HPS. Genomic DNA was extracted from peripheral blood of each individual using standard method. The coding exons and the exon-intron boundaries of *HPS1* gene were amplified and sequenced.

Results: Out of 127 IPF patients, 22 patients had 1 feature and 4 patients had ≥ 2 features of HPS. These 4 patients were genetically analyzed. Genetic analysis identified one compound heterozygous patient with 2 mutations in exon 13, Q397SfsX1 and R439X. Both mutations were not present in our IPF cohort and healthy controls. In the other 3 IPF patients no mutations were found.

Conclusion: Out of 127 IPF patients we identified one compound heterozygous patient with 2 mutations in the *HPS1* gene. HPS is a very rare syndrome, but recognizing is important because of the clinical consequences, particularly when the patient is referred for lung transplantation. Therefore, pulmonologists should be aware of HPS as an underlying cause of pulmonary fibrosis.

Keywords: Hermansky-pudlak syndrome; Idiopathic pulmonary fibrosis; Genetic analysis; Lung transplantation

Abbreviations: HPS: Hermansky-Pudlak Syndrome; IPF: Idiopathic Pulmonary Fibrosis; DNA: Deoxyribonucleic Acid; UIP: Usual Interstitial Pneumonia; CT: Computed Tomography; PCR: Polymerase Chain Reaction; HRM: High Resolution Melting; SIFT: Sorting Intolerant From Tolerant; DIP: Desquamative Interstitial Pneumonia; CVID: Common Variable Immune Deficiency

Introduction

Hermansky-Pudlak syndrome (HPS) is a rare autosomal recessive and genetically heterogeneous disorder. It is characterized by reduced pigmentation of skin, hair and eyes and bleeding diathesis due to absent platelet delta granules [1,2]. Pathogenesis in HPS is caused by disrupted biogenesis or function of lysosome-related organelles due to mutations in organelle related genes [3]. Nowadays, HPS is reported in more than 800 patients. The estimated prevalence of HPS worldwide is 1:500,000 – 1:1,000,000 and therefore almost certainly seems to be underestimated because of mis-diagnosis or un-diagnosis [3]. At our center for interstitial lung diseases, specialized in diagnosing and treating of pulmonary fibrosis, we were visited by a patient with clinical features of HPS. Because of the assumption of underestimation of HPS and the clinical consequences of HPS for further medical treatment, we aimed to establish the prevalence of HPS in a well-described cohort of IPF patients.

To date, mutations in nine different genes (*HPS1*, *AP3B1*, and *HPS3* to *HPS9*) have been identified in human, resulting in subphenotypes HPS-1 to HPS-9 [4]. HPS-1 is the most frequent subphenotype of

HPS. HPS-1, together with HPS-4, is a subunit of the lysosomal complex BLOC-3. Loss of either subunit results in destabilization of the remaining subunits, consequently resulting in clinical features of HPS [3].

One of the clinical features of HPS is pulmonary fibrosis. It is most commonly associated with HPS-1, however pulmonary fibrosis is also reported in cases of HPS-4 and HPS-2 [5]. Pulmonary fibrosis in HPS is characterized by extensive proliferation of type-2 epithelial cells with characteristic foamy swelling, patchy fibrosis with lymphocytic and histiocytic infiltration, and honeycomb changes [6].

However, clinical features of HPS are not always distinct; in fact the severity of the clinical features and the degree of albinism can vary significantly in patients. Therefore, pulmonary fibrosis in unidentified HPS patients with mild extrapulmonary involvement might be easily mistaken for idiopathic pulmonary fibrosis (IPF).

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Received May 20, 2013; **Accepted** June 12, 2013; **Published** June 15, 2013

Citation: van der Vis JJ, ten Klooster L, van Oosterhout MFM, Grutters JC, van Moorsel CHM (2013) The Occurrence of Hermansky Pudlak Syndrome in Patients with Idiopathic Pulmonary Fibrosis—A Cohort Study. J Genet Syndr Gene Ther 4: 141. doi:10.4172/2157-7412.1000141

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IPF is associated with a histopathological or radiological pattern of *usual interstitial pneumonia* (UIP) [7,8]. However, lung histopathology of patients with HPS also typically demonstrates the UIP pattern found in IPF, whereas the radiographic CT findings are not always consistent with UIP [9,10]. The course of pulmonary fibrosis in both IPF and HPS is progressive and lethal. Currently, there is no medical treatment to prolong life in these patients and without lung transplantation they usually die due to rapid clinical deterioration [5,6,11].

Methods

Patient selection

We performed a cohort study of patients diagnosed with IPF enrolled in the Interstitial Lung Disease database of our hospital until March 2013. Patients were included after revision of the diagnosis based on the IPF criteria set by the ATS/ERS/JRS/ALAT [8]. Medical records were screened for clinical features of HPS by two independent investigators (Table 1) [3,4]. In patients who had in addition to pulmonary fibrosis two or more clinical features of HPS DNA sequence analysis of the *HPS1* gene was performed. To determine the frequency of sequence variation, DNA of our Dutch control group of 100 self-reported healthy hospital employees was used. All subjects gave formal written informed consent.

DNA analysis

Genomic DNA was extracted from peripheral blood of each individual using standard method. We amplified and sequenced the coding exons and their exon-intron boundaries of *HPS1* gene (Supplementary Table).

The frequency of sequence variations was determined in the IPF cohort and in 100 controls using high-resolution melting (HRM) analysis (ABI Fast 7500RT; Applied Biosystems, Foster City, CA). The primers used were HPS1 13fw HRM 5'-CCTCTCGGCCCTTACCCTCA-3' and HPS1 12-13 rv primer 5'-CTGCTGTGGACCGGATGTA-3'.

To determine allelic phase, two allele specific PCR's were performed. The forward primer was either specific for the wild type sequence (primer 5'-GGCCCTGGTTCTGTCCCA-3') or for the mutant sequence (primer 5'-GCCCTGGTTCTGTCCA-3'), while the reverse primer was similar in both reactions (primer 5'-CTGCTGTGGACCGGATGTA-3'). Subsequently, both PCR products were sequenced using only the reverse primer in each sequence reaction. Prediction of deleterious amino acid change was performed online at <http://sift.jcvi.org/> using default settings in Sorting Intolerant from Tolerant (SIFT) [12].

Results

IPF cohort

The cohort consisted of 127 patients including 108 male and 19 female. Mean age at time of diagnosis was 64.0 years (SD 10.6 years). Out of 127 patients, 26 patients had besides pulmonary fibrosis, at least one clinical feature consistent with HPS. Most frequently patients had decreased visual acuity, hypercholesterolemia or bleeding diathesis (Table 1). Within this group of 26 patients, 4 patients (PF1, PF2, PF3 and PF4) had 2 or more clinical characteristics of HPS (Flowchart 1). DNA sequence analysis of the *HPS1* gene was performed in these 4 patients and identified two mutations in exon 13, Q397SfsX1 and

Clinical feature	Associated with HPS subtype	No of patients with clinical feature
restrictive lung disease	1, 2, 4	127
variable pigment dilution	1, 2, 4, 7-9	3
decreased visual acuity	1-4, 9	11
nystagmus	1-9	1
hemorrhage, prolonged bleeding, easy bruising	1-8	7
granulomatous colitis	1, 4	1
conductive hearing loss	2	1
neutropenia, immunodeficiency	2	1
dysplastic acetabulae	2	0
poor balance	2	0
hypercholesterolemia	5	8

Table 1: Overview of clinical features of HPS with associated subtype in our IPF cohort.

cDNA position*	Site of variant	Variant name^	Consequence	Patient	rs number
c.297C >T	exon 5	p.Thr99=	synonymous	PF4	rs11539873
c.399-35G >A	intron 5			PF2	rs11591594
c.507+61C >G	intron 6			PF1, PF4	rs1886728
c.636C >T	exon 7	p.Leu212=	synonymous	PF1, PF3	rs1801287
c.937+78C >T	intron 10			PF4	rs1061134
c.937+88C >T	intron 10			PF4	rs34533614
c.1187delC	exon 13	p.Q397SfsX1	frameshift	PF1	
c.1315C >T	exon 13	p.R439X	stop codon	PF1	
c.1335+48G >A	intron 13			PF4	rs41317034
c.1397+7G >C	intron 14			PF3	rs2296432
c.1397+8G >C	intron 14			PF3	rs2296433
c.1472C >G	exon 15	p.Pro491Arg	nonsynonymous	PF4	rs2296434
c.1808A >G	exon 18	p.Gln603Arg	nonsynonymous	PF4	rs2296436

*based on accession number NM_000195.3

^based on accession number NP_000186.2

Table 2: Identified variants in *HPS1* gene.

R439X mutation in patient PF1. Allele specific PCR revealed that the mutations were located on separate alleles (Figure 1).

The Q397SfsX1 mutation found was a deletion of a cytosine at position c.1187 of the cDNA, predicted to change a glutamine to a serine at codon 397 and causing a frameshift resulting in a stop codon at position 398. The R439X mutation was a thymine to cytosine substitution at position c.1315 of the cDNA resulting in a stop codon at position 439 of the HPS1 protein. Allelic phase analysis determined that each allele carried one of the mutations, meaning that this patient is compound heterozygous.

Genotyping showed that both mutations were not present in the IPF patients or in the healthy control subjects. The summary of sequence variations is given in table 2. In patient PF4 two non-synonymous

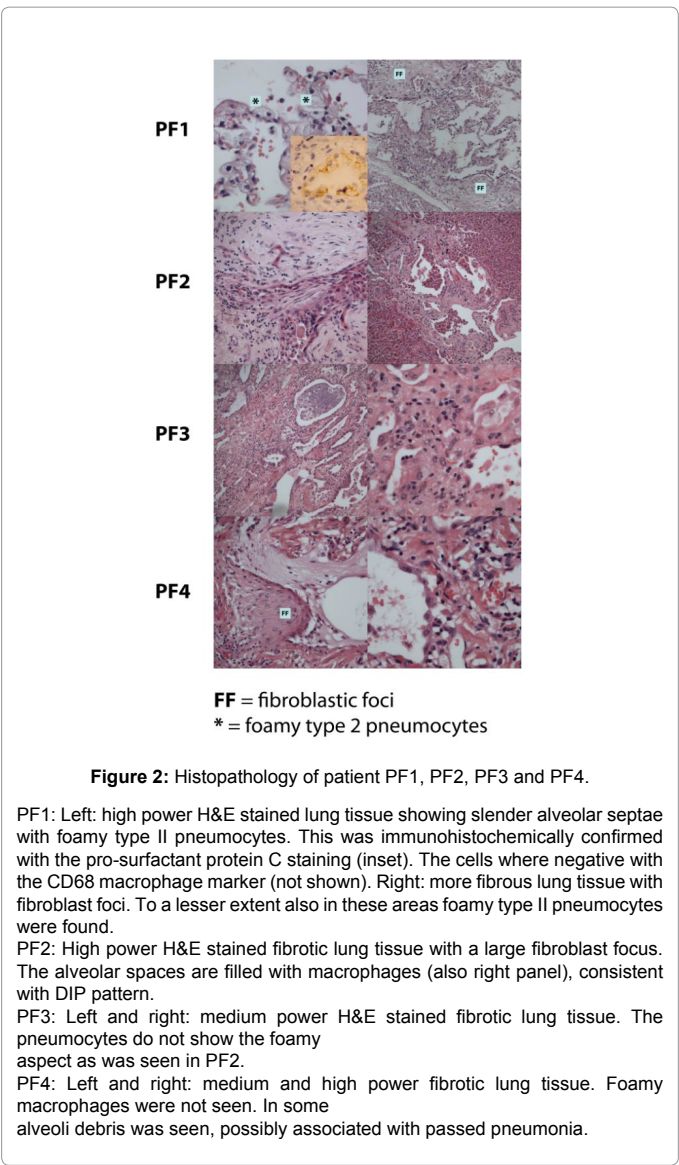
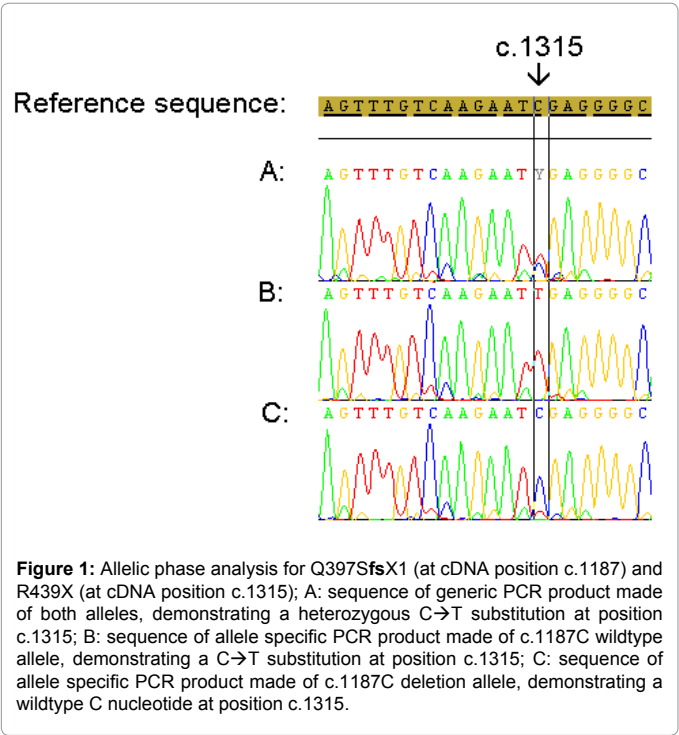
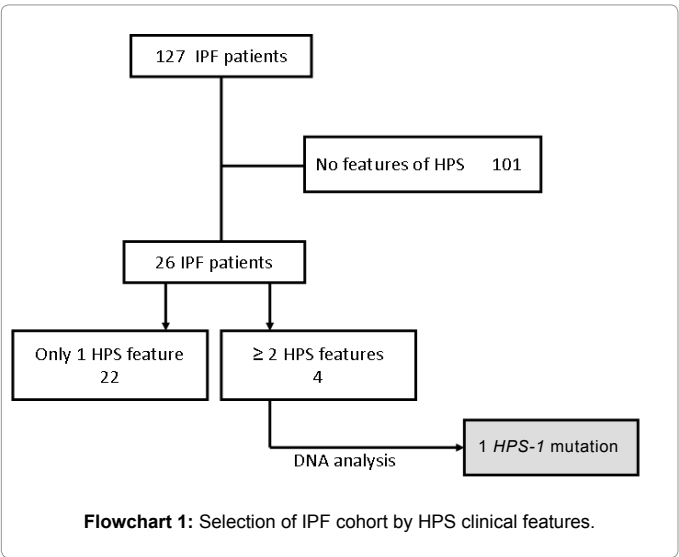


Figure 2: Histopathology of patient PF1, PF2, PF3 and PF4. PF1: Left: high power H&E stained lung tissue showing slender alveolar septae with foamy type II pneumocytes. This was immunohistochemically confirmed with the pro-surfactant protein C staining (inset). The cells were negative with the CD68 macrophage marker (not shown). Right: more fibrous lung tissue with fibroblast foci. To a lesser extent also in these areas foamy type II pneumocytes were found. PF2: High power H&E stained fibrotic lung tissue with a large fibroblast focus. The alveolar spaces are filled with macrophages (also right panel), consistent with DIP pattern. PF3: Left and right: medium power H&E stained fibrotic lung tissue. The pneumocytes do not show the foamy aspect as was seen in PF2. PF4: Left and right: medium and high power fibrotic lung tissue. Foamy macrophages were not seen. In some alveoli debris was seen, possibly associated with passed pneumonia.

variants Pro491Arg (rs2296434) and Gln603Arg (rs2296436) were found. SIFT analysis predicted no consequences on protein function for these variants.

Patient characteristics

Patient PF1 is a 62-year-old Caucasian female diagnosed in 2006 with PA-proven idiopathic pulmonary fibrosis. She is a non-smoker and without significant family history for pulmonary diseases. She had a medical history of oculocutaneous albinism, hysterectomy for excessive bleeding, easy bruising, normocytic anemia and actinic keratosis.

She consulted our outpatient clinic in 2008 for expert opinion. She complained about dyspnea and dry cough. Saturation of peripheral oxygen (SpO₂) was 94% and pulmonary function testing demonstrated a vital capacity of predicted of 35% (1.04 L) and a Tiffeneau index of 85%. High-resolution computed tomography showed bronchiectasis, diffuse ground-glass opacities and interstitial fibrosis predominantly in the bases of the lungs, but no presence of honeycombing. Histopathology showed spatial and temporal heterogeneous fibrosis

with florid fibroblast foci, consistent with UIP. Furthermore, foamy type II pneumocytes, confirmed with pro-sp-C staining, were easily found. This is characteristic for HPS (Figure 2). The foamy type II pneumocytes covered normal appearing alveolar septae and, to a lesser extent, fibrotic areas and overlaid fibroblast foci. After suspicion for HPS platelet function tests determined impaired secondary aggregation response and decreased concentration of ADP, ATP and serotonin, which are features of platelet storage pool disease.

Patient PF2 is a Caucasian female diagnosed with IPF in 2002. She is a former smoker and has a distinct pale skin and light-colored hair. She is visually impaired and has severe conductive hearing loss. Furthermore, she had a hysterectomy due to menometrorrhagia. Histopathology was consistent with a usual interstitial pneumonia (UIP) pattern, but showed also a remarkable accumulation of macrophages in alveolar spaces, consistent with desquamative interstitial pneumonia (DIP). Foamy type II pneumocytes were not present.

Patient PF3 was a Caucasian male diagnosed in 1999 with IPF for which he received unilateral lung transplantation in 2005. Seven years post-lung transplantation he died at the age of 70 years because of metastasized atypical fibroxanthoma. Besides the restricted lung function due to IPF, he had an impaired visual function and hypercholesterolaemia. Histopathology was consistent with UIP. Foamy type II pneumocytes were not present.

Patient PF4 was a Caucasian female with a medical history of hysterectomy and common variable immune deficiency (CVID). She was diagnosed with IPF in 1999 and listed for unilateral lung transplantation in 2005. She died 8 months after listing at the age of 58 years. Histopathology showed a pattern consistent with UIP and in addition some alveoli showed debris which might indicate passed pneumonia. Foamy type II pneumocytes were not present.

Discussion

The prevalence of HPS seems to be underestimated because clinical features of HPS are not always very distinct which can result in misdiagnosis or un-diagnosis. Pulmonary fibrosis is one of the clinical features of HPS and has several similarities to IPF. We conducted a cohort study and we identified one compound heterozygous patient with two mutations in the *HPS1* gene.

The two identified mutations were Q397SfsX1 and R439X. Both mutations cause an early stop codon resulting in a truncated protein, like most of the *HPS1* gene mutations reported to date. With allelic phase discrimination we showed that patient PF1 is compound heterozygous, so no functional HPS-1 will be produced. The Q397SfsX1 mutation was previously reported in patients with HPS-1 of Caucasian origin [13]. Frequency analysis in 100 healthy controls of Dutch origin showed no carriership for this mutation, suggesting this mutation is not common in the Dutch population.

Furthermore, we found two non-synonymous variants in PF4, Pro491Arg (rs2296434) and Gln603Arg (rs2296436). These variants have previously been described in literature and are considered to be non-pathologic DNA polymorphisms [13,14]. Indeed SIFT analysis predicted no consequences on protein function for these variants.

It should be noted that we screened the study population for all clinical features identified in the different subtypes of HPS-1 to HPS-9, however the analysis has concentrated on HPS-1. Although pulmonary fibrosis is most commonly associated with HPS-1, it is also reported in a few cases of HPS-2. HPS-2 is caused by mutations in the *AP3B1* gene.

HPS-1 and HPS-2 have similar clinical features, however immune-related comorbidities are specifically associated with HPS-2 [13,15]. Patient PF4 was known with common variable immunodeficiency (CVID). CVID is an autosomal dominant or recessive inherited disorder affecting the humoral immune system resulting in increased susceptibility to infections and diminished responses to protein and polysaccharide vaccines [16]. To investigate the possibility that this patient had HPS-2 we also sequenced the coding regions of the *AP3B1* gene, but no mutation was found (data not shown).

This study shows that specific clinical features can identify patients suspected for HPS. Out of 127 IPF patients only 4 patients had 2 or more clinical features of HPS besides pulmonary fibrosis and 1 patient could be diagnosed with the syndrome. Genetic analysis should be considered in these patients to exclude HPS as an underlying cause of pulmonary fibrosis. However, in general these patients undergo extensive clinical tests, whereas targeted genetic analysis is fast and simple and can easily establish the diagnosis. It illustrates, due to increasing knowledge of genetic disorders, that cooperation between the physician and geneticist is in favor of the patient. Notwithstanding its limitations of a retrospective study design, this study suggests that increased awareness of clinical features of HPS followed by gene specific analysis, can adequately diagnose these patients.

Although HPS is a rare syndrome, because of the clinical consequences physicians should be aware that it can be the underlying cause of pulmonary fibrosis.

This is particularly important when HPS patients have severe pulmonary fibrosis and are in need of lung transplantation. In the past lung transplantation in patients with HPS has been considered not possible due to bleeding diathesis; however successful lung transplantation is reported for the first time in 2005 [17]. Therefore, lung transplantation is considered to be a treatment option in HPS, although careful assessment of bleeding risks and of pre-operative and intra-operative precautions should be taken.

To our knowledge this is the first study that investigates the prevalence of HPS1 in an IPF cohort. Further investigation is needed for better determination of the occurrence of HPS and to increase awareness for HPS among physicians worldwide. Moreover, we would like to emphasize that due to advancing knowledge, cooperation between chest physicians and clinical geneticists should be encouraged.

Acknowledgment

The authors want to thank P. van der Wagt, M. Baarse, J. Egthuisen, N. de Velde, T. Li, J. Ham, L. Clarisse, S. van den Oever and G. d'Aguiar, students of Hogeschool Leiden Molecular Life Sciences for technical assistance.

This research was enabled by a GlaxoSmithKline BV research grant for the investigation of genetic factors predisposing to interstitial lung disease.

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