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Pulmonary Function and High-Resolution CT Findings in Patients With an Inherited Form of Pulmonary Fibrosis, Hermansky-Pudlak Syndrome, Due to Mutations in *HPS-1**

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Objective: **To describe and correlate pulmonary function and high-resolution CT (HRCT) scan scores in individuals with a high risk for development of pulmonary fibrosis, *ie*, Hermansky-Pudlak syndrome (HPS) patients with mutations in the *HPS-1* gene.**

Design: **Cross-sectional analysis of consecutive, eligible patients.**

Patients: **Thirty-eight HPS inpatients at the National Institutes of Health Clinical Center with *HPS-1* mutations.**

Results: **Thirty-seven patients were Puerto Rican and exhibited the typical 16-base pair (bp) duplication in exon 15 of *HPS-1*. One non-Puerto Rican was homozygous for a different mutation (intervening sequence 17 −2 A→C) previously reported in the *HPS-1* gene; he died at age 35 of pulmonary insufficiency. For the 23 patients who had pulmonary symptoms, the mean age of onset was 35 years. For all 38 patients (mean age, 37 ± 2 years), the mean FVC was 71% of predicted; the mean FEV_1, 76%; mean total lung capacity (TLC), 72%; mean vital capacity (VC), 68%; and mean diffusing capacity of the lung for carbon monoxide (DLCO), 72%. When patients were grouped according to the extent of their reduction in FVC, the other four pulmonary function parameters followed the FVC. Seventeen patients had abnormal chest radiographs, and 31 (82%) had abnormal HRCT scans of the chest, for which a scoring system of 0 (normal) to 3 (severe fibrosis) is presented. The mean ± SEM HRCT score for 38 patients was 1.30 ± 0.17. HRCT scores correlated inversely with FVC and DLCO.**

Conclusions: **Mutations in the *HPS-1* gene, whether or not they involve the typical 16-bp duplication seen in Puerto Rican patients, are associated with fatal pulmonary fibrosis. In affected patients, the FVC, FEV_1, TLC, VC, and DLCO fall in concert, and this functional deficit correlates with HRCT scan evidence of progression of interstitial lung disease.**

(CHEST 2000; 117:129–136)

Key words: albinism; CT scans; pulmonary fibrosis; pulmonary function tests

Abbreviations: cDNA = complementary DNA; DLCO = diffusing capacity of the lung for carbon monoxide; HPS = Hermansky-Pudlak syndrome; HRCT = high-resolution CT; IVS = intervening sequence; NIH = National Institutes of Health; PCR = polymerase chain reaction; TLC = total lung capacity; VC = vital capacity

Hermansky-Pudlak syndrome (HPS) is an autosomal recessive disease characterized by oculocutaneous albinism, a platelet storage pool deficiency, and lysosomal accumulation of ceroid lipofuscin.[1,2] The albinism manifests as congenital nystagmus, iris transillumination, decreased visual acuity, and widely variable hypopigmentation of the skin and hair.[3,4] The platelet storage pool deficiency causes bruising and prolonged bleeding due to an impaired secondary aggregation response; electron microscopic evidence of absent platelet dense bodies provides the *sine qua non* for diagnosing HPS.[5] Ceroid lipofuscin consists of a lipid-protein complex of unknown composition thought to accumulate in cellular lysosomes and believed to cause pulmonary fibrosis[6,7] and granulomatous colitis[8,9] in some patients. In affected individuals, the pulmonary fibrosis typically leads to death in the fourth or fifth decade.[2] Although HPS occurs worldwide, it is most common in northwest

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Manuscript received May 13, 1999; revision accepted August 10, 1999.

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Reprinted from CHEST
Vol. 117, p. 129-136, January 2000 Issue

Puerto Rico, where its frequency is 1 in 1,800[10] owing to a founder effect.

In 1995, a gene causing HPS was mapped to chromosome 10q23.1–23.3 by linkage analysis in the Puerto Rican population.[11,12] In 1996, the gene, now referred to as *HPS-1*, was identified and sequenced,[13] and its intron/exon organization was later characterized.[14] *HPS-1* has 20 exons coding for a 700-amino acid, 79.3-kd protein that is ubiquitously expressed but has no recognized function and no homology to known proteins. To date, 12 different *HPS-1* mutations have been identified among Puerto Ricans and non-Puerto Ricans.[13,15–18] A homozygous 16-base pair (bp) duplication in exon 15 of *HPS-1* has been found in all HPS patients from northwest Puerto Rico but in no non-Puerto Rican patients.[13,15,16,19] The absence of *HPS-1* mutations in *bona fide* HPS patients points to locus heterogeneity in both the Puerto Rican[19] and non-Puerto Rican[15,16] populations.

We previously demonstrated that Puerto Rican patients homozygous for the 16-bp duplication carry an increased risk for developing restrictive pulmonary disease.[20] To further investigate the pulmonary fibrosis associated with HPS, we performed pulmonary function studies and high-resolution CT (HRCT) scans of the chest in 38 patients with mutations in *HPS-1*. Pulmonary function results were correlated with radiographic evidence of fibrosis, and the natural history of pulmonary dysfunction in patients with *HPS-1* mutations was documented. We also provide evidence that *HPS-1* mutations in general, rather than homozygosity for the 16-bp duplication specifically, constitute the risk factor for pulmonary fibrosis.

Materials and Methods

Patients

All the subjects were enrolled in a protocol approved by the National Institute of Child Health and Human Development Institutional Review Board and all gave written informed consent. HPS was diagnosed based on the presence of oculocutaneous albinism and a bleeding diathesis; the diagnosis was confirmed on molecular grounds. To be included in this data analysis, patients were required to be adults admitted to the National Institutes of Health (NIH) Clinical Center, to have mutations in *HPS-1*, and to have undergone an HRCT scan of the lung. Thirty-eight individuals met these criteria between January 1996 and June 1998.

Molecular Studies

Genomic DNA, extracted from peripheral leukocytes or fibroblasts using a standard procedure,[21] served as a template for polymerase chain reaction (PCR) amplification to detect the 16-bp duplication in exon 15 of *HPS-1*. Primers and conditions were as previously described.[13,15,19] When electrophoresed in 3% agarose and stained with ethidium bromide, the 285-bp product of the duplication patients was easily distinguishable from the normal, 269-bp product.

Both complementary DNA (cDNA) and genomic DNA were employed to detect the *HPS-1* mutation in the non-Puerto Rican patient. Reverse transcription PCR was performed after total RNA extraction from cultured fibroblasts using Trizol reagent (Life Technologies, Inc; Grand Island, NY). cDNA was synthesized by reverse transcription using 5 μg of total RNA, according to the protocol of the manufacturer (GIBCO-BRL; Grand Island, NY). The 2.1-kilobase coding region of the *HPS-1* cDNA was amplified using primers 5′-TGCAGCCCTTTCTGAACCTCTG-3′ (forward) and 5′-GAACAGTGGCAAGCAAGGGTG-3′ (reverse). PCR amplification was performed using 2 μL of the first stand cDNA, 1× PCR buffer, 1.5 mM $MgCl_2$, 200 μmol/L dGTP, 200 μmol/L dATP, 200 μmol/L dTTP, 200 μmol/L dCTP, 0.2 μmol/L primers, and 2.5 U *Taq* DNA polymerase in a final volume of 50 μL. After an initial denaturation step at 94°C for 4 min, PCR amplification was conducted for 35 cycles with a denaturation step at 94°C for 30 s, annealing at 55°C for 40 s, extension at 72°C for 2.5 min, and a final elongation step at 72°C for 10 min. Sequencing was performed using primers reported in Table 1 of the publication by Hazelwood et al,[19] specifically, HP-1 (reverse), HP-2 (forward), HP-3 (forward), HP-4 (forward), HP-5 (forward), HP-6 (forward), and HP-7 (forward). Genomic DNA also served as a template for PCR amplification of the individual exons of *HPS-1*. The primers and conditions for genomic DNA were as previously described.[14–16]

Direct, automated DNA sequencing was performed using the ABI Prism dRhodamine Terminator Cycle Sequencing Kit (Applied Biosystems; Foster City, CA) according to the manufacturer's recommendations.

Pulmonary Function Tests

Pulmonary function was assessed as previously described,[22] using well-established normal values.

HRCT of the Chest

HRCT of the chest was performed on a GE Hispeed Advantage scanner (GE Medical Systems; Waukesha, WI) with the patient prone. The thorax was divided into upper, middle, and lower segments, and HRCT findings in each segment were recorded. HRCT scores were based on the following scheme: 0 = normal CT; 1 = mild disease (1 to 15 thickened interlobular septa per segment, one to five patches of reticulation, subpleural cysts, and pleural/parenchymal scars); 2 = moderate disease (moderate reticulation, peribronchovascular thickening, traction bronchiectasis, tracheal retraction); 3 = severe disease (areas of parenchymal consolidation, diffuse areas of peribronchovascular thickening, traction bronchiectasis, and patches of reticulation). Examples of HRCT scans corresponding to each score are shown in Figure 1. Two physicians (M.B. and N.A.A.) assigned CT scores at increments of 0.5 in a masked fashion, and the mean value was designated the subject's HRCT score. The mean difference in scores of the two readers was 0.5, and the inter-reader correlation coefficient was 0.77. The intra-reader correlation coefficient was 0.92.

Statistical Analysis

The lines in Figure 2 were determined using the least-squares method to calculate the best straight line fit. Routine definitions of the SEM and correlation coefficients were employed.