GENETIC DEFECTS AND CLINICAL CHARACTERISTICS OF PATIENTS WITH A FORM OF OCULOCUTANEOUS ALBINISM (HERMANSKY–PUDLAK SYNDROME)

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ABSTRACT

Background Hermansky–Pudlak syndrome is characterized by oculocutaneous albinism, a storage-pool deficiency, and lysosomal accumulation of ceroid lipofuscin, which causes pulmonary fibrosis and granulomatous colitis in some cases. All identified affected patients in northwest Puerto Rico are homozygous for a 16-bp duplication in exon 15 of a recently cloned gene, *HPS*. We compared the clinical and laboratory characteristics of these patients with those of patients without the 16-bp duplication.

Methods Forty-nine patients — 27 Puerto Ricans and 22 patients from the mainland United States who were not of Puerto Rican descent — were given a diagnosis on the basis of albinism and the absence of platelet dense bodies. We used the polymerase chain reaction to determine which patients carried the 16-bp duplication.

Results Twenty-five of the Puerto Rican patients were homozygous for the 16-bp duplication, whereas none of the non–Puerto Rican patients carried this mutation. Like the patients without the duplication, the patients with the 16-bp duplication had a broad variation in pigmentation. Nine of 16 adults with the duplication, but none of the 10 without it, had a diffusing capacity for carbon monoxide that was less than 80 percent of the predicted value. High-resolution computed tomography in 12 patients with the 16-bp duplication revealed minimal fibrosis in 8, moderate fibrosis in 1, severe fibrosis in 1, and no fibrosis in 2. Computed tomography in eight patients without the duplication revealed minimal fibrosis in three and no fibrosis in the rest. Inflammatory bowel disease developed in eight patients (four in each group) between 3 and 25 years of age.

Conclusions The 16-bp duplication in exon 15 of *HPS*, which we found only in Puerto Rican patients, is associated with a broad range of pigmentation and an increased risk of restrictive lung disease in adults. (N Engl J Med 1998;338:1258-64.)

HERMANSKY–PUDLAK syndrome, an autosomal recessive disorder, consists of oculocutaneous albinism, a storage-pool deficiency, and lysosomal accumulation of ceroid lipofuscin.[1-3] The albinism causes congenital nystagmus, a visual acuity approximating 20/200,[4] transillumination of the iris, and mild-to-striking dilution of skin and hair pigmentation. The storage-pool defect arises from the absence of platelet dense bodies, whose contents (adenosine diphosphate, adenosine triphosphate, calcium, and serotonin) trigger the secondary aggregation response of platelets.[2] Patients with Hermansky–Pudlak syndrome have easy bruisability of soft tissues and prolonged bleeding after dental extraction and surgical procedures; prophylaxis with desmopressin can be effective,[5] and avoidance of aspirin products is essential. The accumulation of ceroid lipofuscin, an amorphous lipid–protein complex, is associated with pulmonary fibrosis[6,7] and granulomatous colitis.[8] The pulmonary disease begins with a restrictive component and progresses inexorably to death, usually in the fourth or fifth decade.[2]

Most information concerning Hermansky–Pudlak syndrome has been obtained from the study of patients in northwest Puerto Rico, where the gene frequency approximates 1 in 18 and more than 300 persons are affected.[9] Linkage analysis of Puerto Rican families mapped a gene causing Hermansky–Pudlak syndrome to chromosome 10q23.[10] The gene, *HPS*, has 20 exons coding for a 79.3-kd protein of 700 amino acids whose function is unknown and that is not homologous to any known proteins.[11] Thus, the actual gene product is not known. In the report describing *HPS*,[11] all Puerto Rican patients who were studied were homozygous for a 16-bp duplication in exon 15, reflecting a founder effect. A Japanese patient whose parents were consanguineous had a single-base duplication in codon Ala441, and six Swiss patients and one Irish patient were homozygous for a single-base duplication in codon Pro324.[11]

The availability of molecular genotyping has enabled us to document the phenotype associated with homozygosity for the 16-bp duplication. We also determined its frequency among non–Puerto Rican patients and compared the clinical and laboratory characteristics of patients with the 16-bp duplication and those without the duplication.

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Reprinted from *The New England Journal of Medicine*
338:1258-1264 (April 30), 1998

METHODS

Patients

Forty-nine patients ranging in age from 3 to 54 years were enrolled in the study, which was conducted between November 1995 and February 1997. Hermansky–Pudlak syndrome was diagnosed on the basis of oculocutaneous albinism and a storage-pool deficiency (the absence of platelet dense bodies on electron microscopy)[12] in all but two patients. These two patients had albinism and were siblings of a patient who met the diagnostic criteria. In the case of 41 patients, electron microscopy of the platelets was performed by Dr. James G. White (University of Minnesota) before the study. Documentation of ceroid lipofuscinosis was not required for the diagnosis.

Twenty-two of the patients (from 17 families) lived in the mainland United States and were neither native Puerto Ricans nor of Puerto Rican descent; two of these patients have been described previously.[13,14] The other 27 patients (from 22 families) were Puerto Ricans and included children who were similar in age to the non–Puerto Rican children. Some of the Puerto Rican patients could not travel to the National Institutes of Health Clinical Center for testing because of severe pulmonary disease. We have previously described 2 of the Puerto Rican patients[15]; as many as 19 others were identified by the late Dr. Carl Witkop and may have been described previously.[3,6,9]

The protocol was approved by the institutional review board of the National Institute of Child Health and Human Development, and all patients or their parents provided written informed consent.

Clinical and Laboratory Evaluations

The best corrected visual acuity of patients six years old or older was measured with the Early Treatment Diabetic Retinopathy Study chart and recorded as the Snellen equivalent. Photographs of the transillumination of the fundus and iris were taken in each patient to show the degree of pigmentation.

Glomerular function was estimated according to the equation of Schwartz et al.[16]: 0.55 × height (in centimeters) ÷ serum creatinine (in milligrams per deciliter) = creatinine clearance (in milliliters per minute per 1.73 m^2). Urine samples were also obtained from 45 patients, and the clearance values calculated on the basis of urine and serum creatinine concentrations supported the findings calculated with the use of this equation.

Renal tubular dysfunction was quantified with the Fanconi's syndrome index. In this index the daily urinary excretion of 21 amino acids is measured by ion-exchange chromatography[17] and expressed as micromoles per kilogram of body weight per day. The greater the index, the more severe the defect in tubular reabsorption. At least two 24-hour urine collections of reasonable volumes were used to calculate the index. Plasma lipids were assayed with the Gilchem reagent system (Gilford Diagnostics, Cleveland) in blood obtained from the patients after an overnight fast.[17]

Molecular Studies

To assess samples for the 16-bp duplication, DNA was extracted from lymphocytes.[18] A 269-bp fragment spanning exon 15 of *HPS* was amplified by the polymerase chain reaction (PCR) in a 50-μl reaction mixture containing 50 mM potassium chloride, 1.5 mM magnesium chloride, 5 mM TRIS (pH 8.3), 200 mM of each deoxynucleoside triphosphate, 0.01 percent gelatin, 0.6 mM primers (5′GATGGTCCACAAAGGACGAG3′ and 5′GCGTGAAGGAAGTACGGGCC3′), 2.5 U of *Taq* polymerase, and 500 ng of template DNA. After an initial period of denaturation at 94°C for 2 minutes, amplification was performed for 30 cycles consisting of 1 minute of denaturation at 94°C, 30 seconds of annealing at 60°C, 1 minute of extension at 72°C, and a final 10-minute period of elongation at 72°C. PCR products were subjected to electrophoresis on 2 percent agarose gel and stained with ethidium bromide.

Statistical Analysis

Student's t-test with two-tailed P values, Fisher's exact test with a chi-square distribution, and the Wilcoxon rank-sum test were used to analyze the data.[19]

RESULTS

The 16-bp Duplication

Of the 49 patients, all 25 who were homozygous for the 16-bp duplication in exon 15 of the *HPS* gene (Fig. 1) were from northwest Puerto Rico. The other 2 Puerto Rican patients,[15] as well as all 22 of the non–Puerto Rican patients, had no alleles containing the duplication. The two groups of patients were analyzed separately. Of the 25 patients with the 16-bp duplication, 13 were male and 12 were female (mean [±SD] age, 24±15 years). Of the 24 patients without the duplication, 11 were male and 13 were female (mean age, 19±13 years).

Pigmentation

Both groups of patients had hypopigmentation, with skin color ranging from tan to light and hair color from brown to white (Fig. 2, top panels). The degree of iris pigmentation, as assessed by transillumination (Fig. 2, middle panels), and retinal and choroid pigmentation (Fig. 2, bottom panels) correlated poorly with the degree of skin and hair pigmentation. The extent of pigmentation of the iris and fundus varied considerably. One of the Puerto Rican patients without the 16-bp duplication had nearly complete pigmentation of the iris with negligible transillumination; the other had substantial pigmentation of the iris with diffuse, irregular transillumination.[15]

Ophthalmic Findings

The median visual acuity was similar in the two groups (Table 1). The poorest acuity of the better eye was 20/250 in the 21 patients with the 16-bp duplication in whom it was measured and 20/200 in 20 patients without the duplication. In 12 patients, 8 of whom were homozygous for the 16-bp

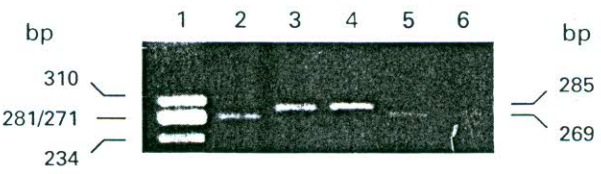

Figure 1. Representative Agarose Gel Showing the 16-bp Duplication in Exon 15 of *HPS*.

PCR amplification yielded a normal 269-bp fragment in control DNA from a subject who did not have Hermansky–Pudlak syndrome (lane 2) and from two patients with the syndrome but without the 16-bp duplication (lanes 5 and 6). A 285-bp fragment was identified in two other patients with the 16-bp duplication (lanes 3 and 4). Lane 1 shows a size marker.