PASPCR Commentary
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Czeck it out: Growing up with Hermansky and Pudlak

My venture into pigmentology began in 1998, when I joined the lab of Dr. Bill Gahl at the National Institutes of Health in Bethesda. I had just received my PhD in Medical Sciences from the University of Nijmegen, Netherlands, and was interested in clinical research that was genetically or biochemically oriented. My search for a short adventure abroad brought me to a disease named after Drs. Hermansky and Pudlak, Czech doctors who in 1959 described two patients with oculocutaneous albinism and a bleeding diathesis (1). Besides the albinism and bleeding, colitis or a fatal pulmonary fibrosis developed in some HPS patients. When I joined the lab, Dr. Gahl had seen approximately 50 HPS patients, mostly Puerto Rican, at the NIH Clinical Center.

1995-1998: *The* HPS gene

A founder-effect of HPS existed in northwest Puerto Rico and in a Swiss mountain village, and these isolates were used for linkage analysis by Scott Wildenberg and Bill Oetting in Dr. Richard King's lab at the University of Minnesota. They mapped "the" HPS gene in 1995 (2). In 1996, further analysis in Dr. Richard Spritz's lab at the University of Wisconsin identified the gene, its genomic organization, a16-base pair duplication in exon 15 in all Puerto-Rican patients, and numerous new mutations in other HPS patients (3). The gene was called simply *HPS*.

My initial task in the Gahl lab seemed just as simple: To screen patients for mutations in this novel gene. Even though we identified new mutations (4), it quickly became apparent that Hermansky-Pudlak syndrome is a heterogeneous disorder: several patients did not have mutations in the *HPS* gene (3,5). At about the same time, Dr. Richard Swanks' lab in Roswell Park (Buffalo, NY) described several mice with albinism and bleeding, all promising models for human HPS (6).

1999: HPS-2

One of those mice, called *pearl*, was found to have mutations in *ap3b1*, a gene coding for the beta 3A subunit of adaptor complex 3 (AP3). This coat protein functions in vesicle formation, and the gene appeared to be a good candidate gene for causing HPS. Indeed, in collaboration with Esteban Dell'Angelica and Juan Bonifacino (NICHD, NIH, Bethesda) our lab found two brothers with

mutations in *AP3B1*, establishing HPS-2 as a distinct disorder (7,8). In 2002, we identified another patient with this HPS subtype (9).

The discovery of an AP3 subunit causing HPS further emphasized that the organelles affected in HPS patients were of the lysosomal lineage, including melanosomes, platelet delta granules, leukocyte lytic granules and fibroblast lysosomes. HPS patients' cells became valuable reagents for studying the biogenesis of lysosome-related organelles, as well as the genes involved in this process (7-11). Not surprisingly, scientific interest in the cell biology of HPS increased exponentially from that point onward.

In 2000, my two years as a postdoctoral fellow at the NIH were concluding, but the work was just beginning to get exciting! I decided to stay a bit longer, and started to pick up more of the cell biology of HPS. I spend some time in Dr. Ray Boissy's lab at the University of Cincinnati and studied cell biological aspects of HPS patients' cells. By using AP3-deficient HPS-2 melanocytes, we found that tyrosinase and tyrosinase-related protein-1 traffic to melanosomes by different routes (12). This served as just one example of how patients' cells can be instructive for cell biology.

2001: HPS-3

About this time, we also realized that even some Puerto Rican patients with HPS did not have the *HPS1* founder mutation of northwest Puerto Rico. One patient from Philadelphia and another from New York City had mild hypopigmentation and traced their ancestry to central rather than northwest Puerto Rico. With Dr. Jorge Toro, we performed homozygosity mapping on affected central Puerto Rican patients and identified a novel gene, *HPS3* (13), whose homolog is the murine gene responsible for *cocao*, one of the HPS mouse models. Additional mutation analysis in non-Puerto Rican HPS patients identified various other *HPS3* mutations, including an Ashkenazi-Jewish founder mutation (14). The mapping data of central Puerto Rican families gave enough information to calculate the time of origin of their *HPS3* founder mutation, i.e., about 5.3 generations ago or between 1880 and 1900. During this time, harsh economic conditions caused a migration from Ciales to a mountainous, more isolated region of central Puerto Rico (Figure 1B). Three of our linkage study families traced their ancestry back to one individual, named Calixto Rivera, who brought his relatives to the central Puerto Rican town of Aibonito to deforest land for tobacco growing. Dr. Yair Anikster in the lab found an old photograph of Aibonito circa 1900 (Figure 1A). In the photo, a little girl with light skin and hair stood out (Figure 1C); could she be *the first affected patient?*

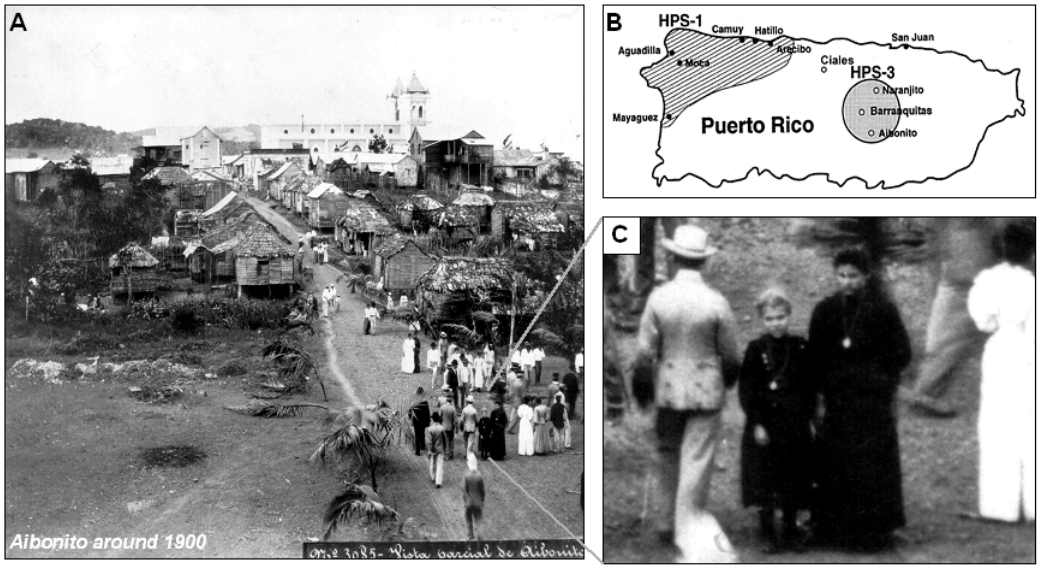

Figure 1: Hermansky-Pudlak syndrome in Puerto Rico. ***A****. Photograph of the town of Aibonito in central Puerto Rico around 1900.* ***B****. Map showing the region of northwest Puerto Rico where HPS-1 is prevalent and the area of central Puerto Rico where the isolate of HPS-3 was found.* ***C****. Enlargement of A.*

2002-2003: HPS-4 through HPS-7

Dr. Gahl's laboratory joined the National Human Genome Research Institute in the summer of 2002 and again I made the decision to stay a little longer and get to know HPS a little better. My move to NHGRI with the lab was based largely on the breadth and depth of learning in the fields of genetics, biochemistry and cell biology that HPS had provided me. By 2003, I continued to work on HPS genetics and cell biology, but as a research fellow in charge of my own research unit within the Medical Genetics Branch of NHGRI. As head of the Cell Biology of Metabolic Disorders Unit, I began investigating metabolic disorders other than HPS, while continuing to be involved in collaborative HPS research in Dr. Gahl's lab.

By now, it appeared that the HPS mouse models described by Dr. Swank's lab (6) were excellent candidates for other HPS-causing genes. Within a short time, the *HPS4* gene was identified as the human homologue of *light ear*, *HPS5* as the human homologue of *ruby eye-2, HPS6* as homologue of *ruby eye*, and *HPS7* as the *sandy* homologue (15). We identified patients with HPS-4, HPS-5, and HPS-6 in our patient population and described the clinical, molecular, and cell biological aspects of each new human subtype in detail (16-19). Recently *HPS8*, corresponding to the *reduced pigment* mouse, was identified (15). This wealth of new HPS subtypes and genes involved in lysosome-related organelle biogenesis promises to reveal further clinical and cell biological insights.

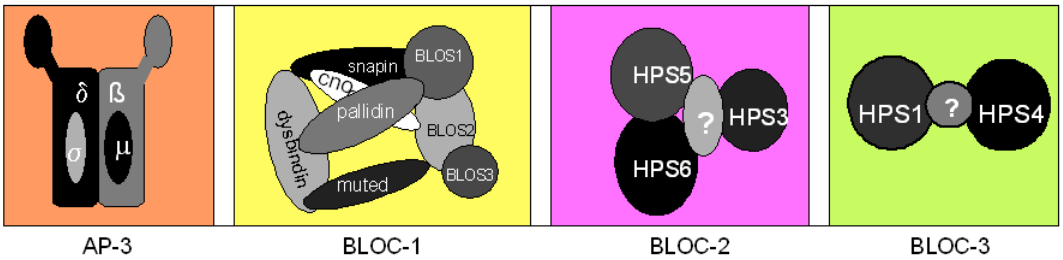

Figure 2: Currently known complexes of HPS-associated proteins.

Current HPS Cell Biology

The eight genes known to cause HPS subtypes each has a mouse counterpart; since there exist additional murine HPS genes, there are likely to be more human HPS genes discovered. Most of the HPS genes are novel, with unknown function and no homology to any known protein or to each other. Three of the mouse HPS genes function in vesicular transport: the Beta3A (mouse *pearl*, human HPS-2) and delta (mouse *mocha*) subunits of AP3, involved in membrane transport and sorting, and *RABGGTA* (mouse *gunmetal*), involved in rab prenylation. Some of the mouse and human proteins are now recognized to interact with each other in so-called BLOCs: Biogenesis of Lysosome Related Organelle Complexes (Figure 2), described in detail by the laboratories of Esteban Dell'Angelica (UCLA), Juan Bonifacino (NICHD, NIH), Richard Spritz (Univ of Colorado), and Richard Swank (Roswell Park, NY). HPS1 and HPS4 interact in BLOC-3; HPS3, HPS5, and HPS6 in BLOC-2; HPS7 and HPS8 are subunits of BLOC-1; and Beta 3A is a subunit of AP3 (see Figure 2) (15,20). By studying deficient HPS patients' cells, we identified clues to the function of the BLOC components (Figure 3). In collaboration with Dr. Ray Boissy, we demonstrated that both BLOC-2 and BLOC-3 deficient melanocytes aberrantly traffic melanocyte-specific proteins (21-23). We also showed a functional clathrin-binding domain in HPS3, and the presence of HPS3 on small clathrin-containing vesicles in the perinuclear region (24). These cell biological studies reveal important details of vesicle trafficking associated with lysosome-related organelle biogenesis. They may also lead to further elucidation of the pathogenesis of Hermansky–Pudlak syndrome and provide the basis for therapeutic interventions for pulmonary fibrosis.

Current Clinical Significance

As of February 2007, Dr. Gahl has admitted approximately 200 HPS patients to the NIH Clinical Center, and we have classified them into 6 subtypes. Delineation of the clinical features associated with each subtype has proven very useful for prognosis. For example, we know that pulmonary fibrosis has occurred only in patients with HPS-1 or HPS-4, and the colitis of HPS occurs more often in these subtypes (25). Cell biological investigations may help find new subtypes, and may explain the pathogenesis of pulmonary fibrosis, allowing for rational therapies; a clinical trial of pirfenidone (26) is ongoing. The colitis of HPS awaits treatment trials, but the neutropenia of HPS-2 appears responsive to granulocyte colony stimulating factor (GCSF) (9).

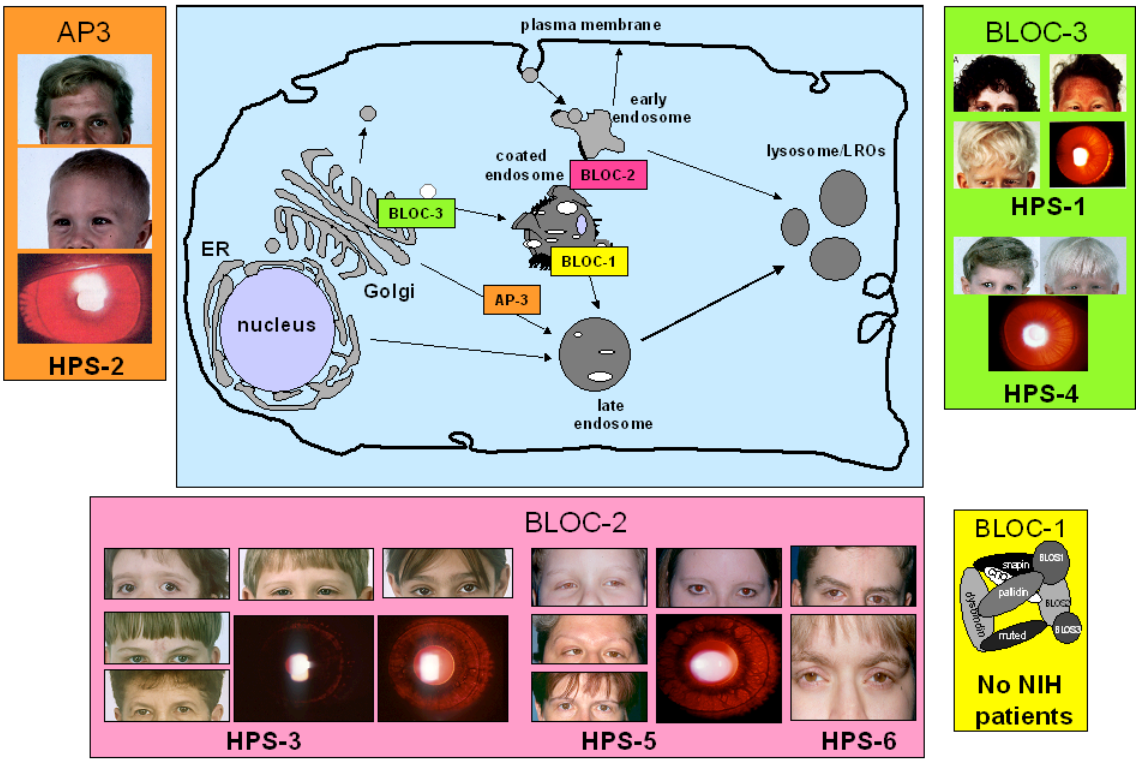

Figure 3: HPS patients' cells assist in identifying clues to the function of the complexes of HPS-associated proteins.

The learning curve in conclusion

My journey into HPS was an adventurous one. Over the last 9 years HPS research has made large leaps forward, as did I. It was very straightforward at first, but led me on many side-roads. During this journey, my knowledge of genetics, biochemistry and cell-biology grew with the knowledge of HPS. I was fortunate to meet, learn from and collaborate with a diversity of experts in the field, dealing with clinical manifestations (Dr. Gahl), cellular aspects of melanosomes (Dr. Boissy) and platelets (Dr. White, Minnesota), genetics (Dr. Yair Anikster, Dr. Swank, the NHGRI community), and advanced cell biology (Dr. Dell'Angelica, Dr. Bonifacino, Dr. Klumperman, Dr. Lambert, and many others). I enjoy being a part of the pigment community, attending PASPCR and ESPCR meetings, and co-chairing the NIH Pigment Research group, known for offering camaraderie and good advice. I grew up professionally with two Czechs, 8 genes, 200 patients, and 9 years of rewarding experiences in the pigment field. I am very grateful for my experience with HPS, and wish that every new postdoc could engage in such a fruitful research project.

Marjan

References:

1) Hermansky F, Pudlak P. Albinism associated with hemorrhagic diathesis and unusual pigmented reticular cells in the bone marrow: report of two cases with histochemical studies. *Blood* 1959; 14:162-169.
2) Wildenberg SC, Oetting WS, Almodovar C, Krumwiede M, White JG, King RA. A gene causing Hermansky-Pudlak syndrome in a Puerto Rican population maps to chromosome 10q2. *Am J Hum Genet* 1995; 57: 755-765.
3) Oh J, Ho L, Ala-Mello S, Amato D, *et al*. Mutation analysis of patients with Hermansky-Pudlak syndrome: a frameshift hot spot in the HPS gene and apparent locus heterogeneity. *Am J Hum Genet* 1998; 62: 593-598.
4) Hermos CR, Huizing M, Kaiser-Kupfer MI, Gahl WA. Hermansky-Pudlak syndrome type 1: gene organization, novel mutations, and clinical-molecular review of non-Puerto Rican cases. *Hum Mutat* 2002; 20: 482.
5) Hazelwood S, Shotelersuk V, Wildenberg SC, Chen D, Iwata F, Kaiser-Kupfer MI, White JG, King RA, Gahl WA. Evidence for locus heterogeneity in Puerto Ricans with Hermansky-Pudlak syndrome. *Am J Hum Genet* 1997; 61: 1088-1094.
6) Swank RT, Novak EK, McGarry MP, Rusiniak ME, Feng L. HPS Mouse models of Hermansky Pudlak syndrome: a review. *Pigment Cell Res* 1998;11: 60-80.
7) Dell'Angelica EC, Shotelersuk V, Aguilar RC, Gahl WA, Bonifacino JS. Altered trafficking of lysosomal proteins in Hermansky-Pudlak syndrome due to mutations in the beta 3A subunit of the AP-3 adaptor. *Mol Cell* 1999; 3: 11-21.
8) Shotelersuk V, Dell'Angelica EC, Hartnell L, Bonifacino JS, Gahl WA. A new variant of Hermansky-Pudlak syndrome due to mutations in a gene responsible for vesicle formation. *Am J Med* 2000; 108: 423-427.
9) Huizing M, Scher CD, Strovel E, Fitzpatrick DL, Hartnell LM, Anikster Y, Gahl WA. Nonsense mutations in ADTB3A cause complete deficiency of the beta3A subunit of adaptor complex-3 and severe Hermansky-Pudlak syndrome type 2. *Pediatr Res* 2002; 51: 150-158.
10) Shotelersuk V, Gahl WA. Hermansky-Pudlak syndrome: models for intracellular vesicle formation. *Mol Genet Metab* 1998; 65: 85-96.
11) Huizing M, Anikster Y, Gahl WA. Hermansky-Pudlak syndrome and related disorders of organelle formation. *Traffic* 2000; 1: 823-835.
12) Huizing M, Saranjarajan R, Strovel E, Zhao Y, Gahl WA, Boissy RE. AP-3-dependent vesicles carry tyrosinase, but not TRP-1, in cultured human melanocytes. *Mol Biol Cell* 2001; 12: 2075-2085.
13) Anikster Y, Huizing M, White J, Shevchenko YO, Fitzpatrick DL, Touchman JW, Compton JG, Bale SJ, Swank RT, Gahl WA, Toro JR. Mutation of a new gene causes a unique form of Hermansky-Pudlak syndrome in a genetic isolate of central Puerto Rico. *Nat Genet*2001; 28: 376-380.
14) Huizing M, Anikster Y, Fitzpatrick DL, Jeong AB, D'Souza M, Rausche M, Kaiser-Kupfer MI, White JG, Toro JR, Gahl WA. Hermansky-Pudlak syndrome type 3 in Ashkenazi Jews and other non-Puerto Rican patients with hypopigmentation and platelet storage pool deficiency. *Am J Hum Genet*2001; 69: 1022-1032.
15) Wei ML. Hermansky-Pudlak syndrome: a disease of protein trafficking and organelle function. *Pigment Cell Res* 2006; 19: 19-42.

16) Anderson PD, Huizing M, Claassen DA, White J, Gahl WA. Hermansky-Pudlak syndrome type-4 (HPS-4): Clinical and molecular characteristics. *Hum Genet* 2003; 113: 10-17.
17) Huizing M, Hess R, Dorward H, Claassen DA, Helip-Wooley A, Kleta R, Kaiser-Kupfer MI, White JG, Gahl WA. Cellular, molecular, and clinical characterization of new patients with Hermansky-Pudlak syndrome type 5. *Traffic* 2004; 5: 711-722.
18) Hess R, Claassen DA, White J, Gahl WA, Huizing M. New patients with Hermansky-Pudlak syndrome type 5 and type 6. *Pigment Cell Res* 2005; 18 (s1), 54-84 (PP041).
19) Schreyer-Shafir N, Huizing M, Anikster Y, Nusinker Z, Bejarano-Achache I, Maftzir G, Resnik L, Helip-Wooley A, Westbroek W, Gradstein L, Rosenmann A, Blumenfeld A. A new genetic isolate of Hermansky Pudlak Syndrome type 6: Clinical, Molecular and Cellular characteristics. *Hum Mutat* 2006; 27: 1158.
20) Dell'Angelica EC. The building BLOC(k)s of lysosomes and related organelles. *Curr Opin Cell Biol* 2004; 16: 458-464.
21) Boissy RE, Richmond B, Huizing M, Helip-Wooley A, Zhao Y, Koshoffer A, Gahl WA. Melanocyte specific proteins are aberrantly trafficked in melanocytes of Hermansky-Pudlak syndrome type 3. *Am J Pathol* 2005; 166: 231-240.
22) Richmond B, Huizing M, JKnapp J, Koshoffer A, Zhao Y, Morris R, Gahl WA, Boissy RE. Hermansky-Pudlak syndrome types 1-3 melanocytes express distinct defects in cargo trafficking. *J Invest Dermatol* 2005; 124: 420-427.
23) Helip-Wooley A, Boissy RE, Westbroek W, Dorward H, Koshoffer A, Huizing M, Gahl WA. Improper trafficking of melanocyte-specific proteins in Hermansky-Pudlak syndrome type-5. *J Invest Dermatol* 2007, Feb 15 [Epub ahead of print].
24) Helip-Wooley A, Westbroek W, Dorward H, Mommaas M, Boissy R, Gahl WA, Huizing M. Hermansky-Pudlak syndrome type-3 protein interacts with clathrin traffics lysosome-related organelles. *BMC Cell Biol* 2005; 6: 33.
25) Hussain N, Quezado M, Huizing M, Geho D, White JG, Gahl W, Mannon P. Intestinal Disease in Hermansky-Pudlak Syndrome: Occurrence of colitis and relation to genotype. *Clin Gastro Hepatol* 2006; 4: 73-80.
26) Gahl WA, Brantly M, Troendle J, Avila NA, Padua A, Montalvo C, Cardona H, Calis KA, Gochuico B. Effect of pirfenidone on the pulmonary fibrosis of Hermansky-Pudlak syndrome. *Mol Genet Metab* 2002; 76: 234-242.