Molecular Defects that Affect Platelet Dense Granules

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Abstract

Platelet dense granules form using mechanisms shared by melanosomes in melanocytes and by subsets of lysosomes in more generalized cells. Consequently, disorders of platelet dense granules can reveal how organelles form and move within cells. Models for the study of new vesicle formation include isolated δ-storage pool deficiency, combined αδ-storage pool deficiency, Hermansky-Pudlak syndrome (HPS), Chediak-Higashi syndrome, Griscelli syndrome, thrombocytopenia absent radii syndrome, and Wiskott-Aldrich syndrome. The molecular bases of dense granule deficiency are known for the seven subtypes of HPS, as well as for Chediak-Higashi syndrome, Griscelli syndrome, and Wiskott-Aldrich syndrome. The gene products involved in these disorders help elucidate the generalized process of the formation of vesicles from extant membranes such as the Golgi.

Keywords

Platelet dense granules; storage pool deficiency; Hermansky-Pudlak syndrome; lysosome-related organelles; intracellular vesicle formation

This review concentrates on the dense bodies of platelets, or δ-granules, paying special attention to relevant human disorders. For some such disorders (e.g., Hermansky-Pudlak syndrome [HPS]), the molecular bases have been determined, whereas other dense-granule deficiency states, such as isolated δ-storage pool deficiency (SPD), are distinguished only by their clinical and histological characteristics. Still other diseases, such as Chediak-Higashi syndrome (CHS), Griscelli syndrome (GS), and thrombocytopenia absent radii (TAR) syndrome, may not lack dense bodies entirely, but manifest evidence for reduced number, size, or function of δ-granules. We first describe the dense granule itself and then discuss its disorders in humans.

THE DENSE GRANULE

Contents and Function

Normal human platelets contain three to eight dense granules, each measuring 200 to 300 nm in diameter.[1] Some dense granules possess a clear halo encircling a calcium-containing core that is electron dense on un-stained whole mounts[2] (Fig. 1). Dense granules are also highly osmophilic, appearing dark on transmission electron microscopy.[3] They contain serotonin, adenosine diphosphate (ADP), adenosine triphosphate (ATP), and pyrophosphate. [3,4] Serotonin is actively taken up from the blood by a plasma membrane transporter and accumulates in the slightly acidic (pH 6.1) dense granule. These vesicles contain approximately two thirds of the ATP and ADP in platelets at a molar ratio of 2:3, much less than the ratio in plasma (8:1).[3] Dense granule membranes house the lysosomal membrane proteins LAMP2 and CD63 (granulophysin or LAMP3), but not LAMP1.[5,6] They also contain P-selectin and the integrin $\alpha_{IIb}\beta_3$, which are constituents of the α-granule and plasma membrane.

Upon activation of a platelet, its dense granule membranes fuse with the plasma membrane via the mechanism of soluble *N*-ethymaleimide-sensitive factor attachment protein receptor (SNARE) complex formation.[7] SNARE proteins are inherent components of both the granule membrane and the plasma membrane that recognize each other and form a complex that facilitates docking of the granule to the plasma membrane. This docking does not require ATP,[8] but it does require priming by other proteins, including *N*-ethylmaleimide-sensitive factor and synaptosome-associated proteins (SNAPs), specifically SNAP-23, to form a fusion complex.[7]

With exocytosis, the dense granule's contents are disgorged, and its ADP activates and recruits other platelets.[3] Serotonin causes vasoconstriction and assists in the binding of adhesive proteins to their platelet receptors.[3]

Biogenesis

Like α-granules, dense granules originate from the Golgi complex[9,10] and are transported to the periphery of megakaryocytes via microtubules and actin filaments, which effect proplatelet formation and elongation. Along this path, a dense granule engages with a multivesicular body (MVB), the key storage and sorting compartment involved in the biogenesis of both α- and dense granules.[10,11] There exist different types of MVBs. Type 1 MVBs have many internal vesicles that contain only the α-granule proteins β-thromboglobulin and von Willebrand factor (vWF). Type 2 MVBs have internal vesicles that contain CD63 and also have electron-dense material containing β-thromboglobulin and vWF.[11]

Young megakaryocytes, with an immature demarcating membrane system, have multivesicular bodies but few α- and dense granules.[11,12] As megakaryocytes enlarge, MVBs become fewer, the number of α- and dense granules increases, the demarcating membrane system develops, and the granules are transported into the developing proplatelets.[11,12]

Storage Pool Deficiencies

The term SPD describes a heterogeneous group of congenital bleeding disorders caused by deficiency of granule-bound substances in platelets.[1,13,14]SPD platelets have decreased amounts of ADP, serotonin, calcium, and pyrophosphate.[15] The discovery of platelets with isolated a-granule deficiency (α-SPD or gray platelet syndrome)[16] or deficiency of both α- and dense granules (combined αδ-SPD),[13] expanded the definition of SPD. Currently, δ-SPD defines patients with dense granule defects[13] that are either acquired (as in myeloproliferative or rheumatologic disorders[14]) or congenital. We will deal here only with inherited δ-SPDs.

ISOLATED δ-SPD

δ-SPD results in easy bruising, mucous membrane bleeding, and excessive postoperative and postpartum hemorrhage,[1,13,14,17] which can be exacerbated by aspirin or other antiplatelet agents. Typically, platelet counts are normal[14,17] and the bleeding time is prolonged. The primary aggregation response is normal, but the secondary aggregation wave is absent or muted. In one study, 23% of 106 patients with storage pool deficiency (based on decreased content of adenine nucleotides and serotonin) had completely normal platelet aggregation results.[14]

Diagnosis of δ-SPD requires measurement of dense granule constituents and/or electron microscopy to demonstrate the absence of dense granule-limiting membranes and contents. [13] Adenine nucleotides are reduced, with an increased ratio of ATP to ADP.[13,15] Platelet serotonin is variably reduced[13,15] and lysosomal enzymes are normal.[13,18] Whole-mount electron microscopy recognizes calcium,[18] but it cannot differentiate the absence of a dense granule from the absence of its calcium. Both full and empty δ-granules take up the fluorescent dye quinacrine (mepacrine) which can be used to quantitate dense granules by fluorescent microscopy.[19,20]

The causative gene, basic defect, and mode of inheritance for δ-SPD remain unknown, although evidence for autosomal dominant inheritance has been presented.[1,13,17,19] Congenital δ-SPD appears to be underdiagnosed. Nieuwenhuis et al[14] reviewed 390 patients with a bleeding tendency; among the 145 with a prolonged bleeding time and normal platelet counts, 27 (18%) had congenital δ-SPD. δ-SPD patients have a propensity to develop myeloproliferative disorders.[21]

Patients with δ-SPD should be instructed to avoid aspirin and other antiplatelet agents. Platelet transfusions effectively treat hemorrhage in patients with δ-SPD. Desmopressin normalizes the bleeding time in some patients.[22] Oral and topical antifibrinolytic agents such as tranexamic acid and ε-amino caproic acid are useful for treating epistaxis and oropharyngeal bleeding.

COMBINED αδ-SPD

Patients with αδ-SPD have diminution of dense granules and a variable deficiency of α-granules and their constituents.[13,17] The α-granules may be entirely absent from some

platelets but their number may be close to normal in others.[13] αδ-SPD can present in mosaic form, with some platelets having normal and some having decreased α- and dense granules. [23]

Patients with αδ-SPD appear healthy except for their bleeding tendency, which resembles that of δ-SPD and manifests with a prolonged bleeding time.[13,17] Platelet counts are normal but platelet aggregation studies may or may not be normal. Diagnosis requires measurement of dense and α-granule constituents and/or electron microscopy. Lysosomal enzymes of αδ-SPD platelets are normal.

The frequency of αδ-SPD is not known, but the disorder appears to be less common than isolated δ-SPD.[13,17] Among 43 patients with prolonged bleeding times, seven had αδ-SPD. [17] The mode of inheritance appears to be autosomal dominant. The basic defect remains unknown, and no causative gene has been identified.

The *gunmetal* mouse has reduced α- and dense granules as well as disorganized megakaryocyte internal membranes and impaired retention of α-granule proteins. This mouse has mutations in *RabggtA* and defective activity of geranylgeranyl transferase, an enzyme that attaches lipid geranylgeraniol groups to certain rab proteins.[24] Rabs are small guanosine triphos-phatases (GTPases), which split GTP to provide energy for membrane fusion events and for attachment to cytoskeletons. Human αδ-SPD may result from mutations in *RabggtA*.

HERMANSKY-PUDLAK SYNDROME

The bulk of our knowledge concerning dense granule deficiency disorders derives from studies of HPS. This disease, named after two Czechoslovakian pathologists who described the disorder in 1959,[25] consists of oculocutaneous albinism and a platelet SPD. Much of our understanding of HPS derives from the study of a large isolate in northwest Puerto Rico, where ~450 individuals have HPS-1, due to a founder effect.[26,27] However, there are now seven known HPS-causing genes, and seven subtypes of HPS. All are autosomal recessive, and no clinical findings have been reported in heterozygotes. There exist at least 14 HPS mice, and each human subtype is associated with a murine model, leaving seven mice scurrying to find a human counterpart. There exist mutants of *Drosophila* with pigment granule defects due to impaired HPS-related genes, and yeast with defects in vesicle proteins for sorting that affect vesicle formation and trafficking.[28,29]

Clinical Findings

All HPS patients have oculocutaneous albinism, with variable hypopigmentation of hair, skin, and irides.[30,31] In certain HPS subtypes, the hypopigmentation is virtually indiscernible (Fig. 2). Ophthalmic abnormalities are always apparent, however.[27,32] Most patients have congenital nystagmus, and visual acuities range from 20/50 to 20/400. Iris transillumination indicates a finite decrease in iris pigment, and retinal fundi are pale in a scattered pattern. Visual evoked potentials reveal abnormal decussation of optic nerve fibers.

By definition, all HPS patients lack platelet dense bodies,[2] apparently due to a defect in organelle development. Platelet aggregation studies show absence of the secondary wave in response to ADP and epinephrine and an impaired response to collagen.[15] The bleeding time is usually prolonged, and mucus membrane bleeding, bruising, epistaxis, and metromenorrhagia are common.[27] Many patients have received whole blood or platelet transfusions, but death from bleeding complications virtually never occurs.

Some patients with some types of HPS develop pulmonary fibrosis, which is generally fatal between the fourth and sixth decades.[27,33] This may have an inflammatory component[34] and begins as mild restrictive lung disease. Approximately 15% of all patients have granulomatous colitis,[27,35] which may involve any portion of the alimentary tract, but largely affects the colon. The colitis resembles Crohn's disease in histology and response to treatment. A lipid-protein complex of unknown etiology called ceroid lipofuscin has been found in the cells and tissues of many HPS patients, largely Puerto Ricans with HPS-1.[27]

The diagnosis of HPS relies on the finding of oculocutaneous albinism and absent platelet dense bodies on whole-mount electron microscopy.[3,28] Transmission electron microscopy sometimes provides equivocal results. The definition of HPS may expand as we discover defects in the number or size of dense bodies rather than their complete absence.

Treatment of HPS is limited.[27,29] The albinism requires skin protection, and visual aids are critically important. Bleeding can be controlled topically with thrombin and Gelfoam, and intravenous 1-desamino-8-D-arginine vasopressin is used prophylactically for procedures such as tooth extractions or biopsies. Birth control pills regulate menstrual bleeding. For large bleeds or surgeries, platelet or red blood cell transfusions may be required.

HPS Subtypes

The amount of information available for any HPS subtype reflects the number of affected individuals (Table 1). HPS-1 represents the most severe form of the disease. HPS-1 occurs in 1 of every 1800 northwest Puerto Rican citizens and has variable dermatologic[36] and ophthalmologic findings, bleeding complications, colitis, and pulmonary involvement, which might respond to therapy with the investigational drug pirfenidone.[37] HPS-1 patients of Japanese, Swiss, and northern European descent manifest typical phenotypes.

HPS-2 affects only four known patients who had neutropenia and increased childhood infections, oculocutaneous albinism, and bleeding.[38–40] None of the patients has pulmonary fibrosis or colitis but all are too young to inform us whether this subtype is associated with lung disease.

HPS-3 occurs in an isolated region of central Puerto Rico, as well as among Ashkenazi Jews and others.[41] Approximately 25 HPS-3 patients are known. The hypopigmentation is extremely mild, so that occasional patients have been diagnosed with ocular (rather than oculocutaneous) albinism. Ophthalmic involvement is also attenuated, along with the bleeding tendency. Colitis does occur in HPS-3, but pulmonary fibrosis probably does not.

HPS-4 affects ~15 individuals and resembles HPS-1, complete with fatal pulmonary fibrosis. [42,43] HPS-5 has been reported in one person, a 3-year old Turkish boy with a bleeding time

of greater than 7 minutes.[44] We know of four additional patients, none with pulmonary fibrosis; two are ~50 years of age. HPS-6 has been reported in one patient, a 39-year-old Belgian woman with no pulmonary or gastrointestinal symptoms.[44] We have diagnosed two additional patients, but they are too young for us to determine if pulmonary involvement will occur. One case of HPS-7 has been described in a 48-year-old Portuguese woman with albinism and a bleeding time of 13 minutes.[45] She had dyspnea on exertion but normal pulmonary function tests.

Molecular Defects

The *HPS1* gene is 31.5-kb in length, 2103 bp of which comprise its 20 exons (Table 1).[26] The murine gene, *hps1*, is mutated in the *pale ear* mouse.[27] The most frequent *HPS1* mutation is a 16-bp duplication in exon 15 common in northwest Puerto Rico.[26,27] A polymerase chain reaction (PCR)-based assay is available from GeneDx, Gaithersburg, MD. There exist at least 13 other *HPS1* mutations, including deletions, insertions, nonsense, and splice site mutations.[26,46] No genotype or phenotype correlation has appeared in HPS-1. The *HPS1* gene product is a 700-amino acid protein with a molecular weight of 79.3 kd. It is not modified by glycosylation and has no homology to known proteins, although it shares a stretch of amino acids (DKF(L/V)KNRG) with the LYST protein of CHS.

The defective gene in HPS-2 is called *AP3B1*[47] (Table 1), has 27 exons within 292.3 kb of genomic DNA, and transcribes a message of 4.2 kb. Homologous genes are mutated in the *pearl mouse* and in a *Drosophila* mutant called *ruby*. Six AP3B1 mutations have been found in four affected humans, including an in-frame deletion, a missense, and two nonsense mutations.[38,39] The patient with two nonsense mutations is more severely affected than the brothers with the in-frame mutations. *AP3B1* codes for the β3A subunit of AP3, a heterotetrameric complex responsible for vesicle formation from the trans-Golgi network.[38–40,48]

The *HPS3* gene consists of 3921 bp and 17 exons[41] (Table 1). Its mRNA is 4.4 kb, and the murine counterpart is mutated in the *cocoa* mouse. A founder mutation arose in central Puerto Rico in ~1880 to 1890 and consists of a 3904-bp deletion.[41] At least six other *HPS3* mutations have been described, including IVS5 + 1G > C, a splicing mutation found among the Ashkenazi Jews.[49] The *HPS3* gene product has 1004 amino acids with a molecular weight of 113.7 kd, a clathrin binding site, no transmembrane regions, and no known function.

The *HPS4* gene consists of 14 exons and spans 43.9 kb (Table 1). Several transcripts exist; the major one is 4.5 kb in size. The corresponding mouse mutant is *light ear*.[42] At least nine different *HPS4* mutations exist in 15 humans, including insertions, deletions, nonsense, and missense mutations.[42,43] No genotype or phenotype correlation has been demonstrated. The function of the *HPS4* product is unknown, but it appears to interact with the *HPS1* gene product.[50]

The *HPS5* gene spans 43.4 kb of DNA and codes for a 4.8-kb transcript with 23 exons producing a 127.4-kd protein (Table 1). The mouse model is *ruby eye-2*, which has 81% sequence homology with the human gene. Only one mutation has been reported. The *HPS5*

gene product has no transmembrane domains or signal sequences, and its function is unknown.[44]

The *HPS6* gene consists of a single exon of 2.7 kb, the product of which is an 83.0-kd protein of unknown function. The murine model is *ruby eye*.[44]

The gene causing HPS-7, *DTNBP1*, consists of 10 exons spanning 140 kb of DNA. The human transcript is 1.5 kb in size, and murine mutations produce the *sandy* mouse.[45] One human mutation, 307C > T (Q103X), has been identified. The human gene product, called dysbindin, has a molecular mass of 39.5 kd. Dysbindin is a component of the biogenesis of lysosome-related organelles complex-1 (BLOC-1; see the following discussion). It also binds to dystrobrevins, which are components of a dystrophin-associated protein complex in muscle and other cells.[45]

Cell Biology and Gene Function

Megakaryocytes have not been cultured from HPS patients. However, the results of studies of lysosomes in fibroblasts and melanosomes in melanocytes can be applied to dense bodies in platelets. Lysosomes undergo biogenesis in a manner similar to that of lysosome-related organelles (LROs), which include melanosomes and platelet dense bodies; they are formed from the trans-Golgi network and endosomes by processes involving vesicle sorting, budding, targeting, transport, maturation, and fusion.

AP3 functions in LRO biogenesis.[47] In HPS-2 fibroblasts (lacking the β3A subunit of AP3), trafficking of lysosomal membrane proteins LAMP1 and LAMP3 through the plasma membrane is enhanced, suggesting that the plasma membrane provides a default pathway for these proteins that operates when normal AP3 function is blocked.[39,47] In fact, the LAMP3 distribution in fibroblasts of the different HPS subtypes corresponds with the severity of the clinical phenotype. In HPS-2 melanocytes, tyrosinase expression is reduced and limited to multivesicular bodies in the perinuclear region, suggesting that tyrosinase trafficking is mediated by AP3.[51] We infer that the platelets of HPS-2 patients also exhibit defective sorting or trafficking of dense granule proteins. Granulophysin/CD63 and LAMP2 are examples of proteins enriched in the dense body membrane and carrying an AP3 tyrosine sorting signal.[52]

In addition to *AP3B1*, all HPS-causing genes encode novel proteins with no recognizable homology to other proteins. HPS-1 is largely cytoplasmic; a portion is membrane bound.[53] Some HPS proteins interact with each other in oligomeric complexes called BLOCs. For example, the HPS-1 and HPS-4 proteins interact in BLOC-3,[50] which explains the similar phenotype of patients with *HPS1* or *HPS4* defects. Morphologic studies of HPS-1 melanocytes showed similar membranous complexes containing membrane-bound chambers, unpigmented and pigmented melanosomes, irregular deposits of tyrosinase, and granular or amorphous material—presumably the products of missorted platelet dense granule components.[54] Missorting of tyrosine-related protein-1 and tyrosinase was also demonstrated by transfecting normal melanocytes with antisense *HPS1* cDNA.[55]

The HPS-5 and HPS-6 proteins interact in BLOC-2.[44] HPS-5 and HPS-6 patients show a similar, mild clinical phenotype. Their fibroblasts show decreased amounts of LAMP1 and LAMP3 reaching peripheral lysosomes, but no accumulation of LAMPs in the perinuclear area (Fig. 3; Huizing, unpublished results).

The HPS7 protein is a homologue of the murine *sandy* gene.[45] The sandy protein is part of BLOC-1, which also contains the proteins coded for by the *pallid, muted*, and *cappuccino* murine HPS genes.[45,56,57] HPS7, the only component of BLOC-1 to cause HPS in humans, is also called dysbindin, a β-dystrobrevin-binding protein. Dystrobrevins are components of the dystrophin-associated protein complex (DPC) in both muscle and nonmuscle cells.[58] The specific functions of HPS7 in BLOC-1 and of the DPC in fibroblasts, melanocytes, and platelets remain to be determined.

CHEDIAK-HIGASHI SYNDROME

CHS is a rare autosomal recessive disorder characterized by defective platelet dense granules, oculocutaneous albinism, immune deficiency, and progressive neurological dysfunction.[59,60] Many cell types in CHS patients contain giant cytoplasmic inclusions, which are enlarged vesicles.[59]

Clinical Findings

Most CHS patients present in early childhood with severe bacterial infections and succumb to an accelerated phase characterized by hemophagocytic lymphohistiocytosis due to uncontrolled T-cell activation.[59] Approximately 10 to 15% of patients exhibit a milder immune phenotype, survive into adulthood, and develop a progressive neurological syndrome in the third to fifth decades, manifesting ataxia, gait problems, weakness and sensory deficits.[59,60] Rarely, patients who present in childhood with severe infections and a moderate pigment defect escape progression to the accelerated phase.[60] The *beige* mouse is the murine counterpart of CHS patients.

In CHS, platelet counts are normal prior to development of the accelerated phase.[59,61] However, a dense-granule defect causes bleeding and a prolonged bleeding time. CHS patients have an impaired secondary wave of platelet aggregation, with an increased ATP-to-ADP ratio, decreased platelet serotonin, and decreased platelet calcium.[62] Some humans[63] and mink[64] with CHS have a normal number and shape of dense bodies, but other reports indicate absent or markedly reduced dense bodies in CHS platelets from humans[65] as well as mice[66] and cattle.[67] *Beige* mice showed mildly decreased mepacrine-containing granules.

CHS patients can manifest complete oculocutaneous albinism or apparently normal pigmentation.[59] Hair, eyebrows, and eyelashes may have a silvery-gray metallic sheen. Pigment in the hair shaft is distributed in homogeneous small clumps.[59,60]

CHS patients have defective cytotoxic T-cell and natural killer-cell function, decreased chemotaxis of macrophages and polymorphonuclear leukocytes, and delayed intracellular killing.[59] Antigen presentation, which requires appropriate vesicular trafficking, is also

defective. Phagocytic activity is normal but fusion of phagosomes with lysosomes is delayed.

Defective cytotoxic T-cell function results in an inability to turn off activated lymphocytes, especially after Epstein-Barr virus infection.[59] As a result, most patients develop an accelerated phase of nonmalignant lymphohistiocytic infiltration, cytokine overproduction, and multiorgan damage. Response to chemotherapy is poor. Bone marrow transplantation cures the immune deficiency and related lymphohistiocytosis,[59] but does not prevent the progressive neurological defect.

The *LYST* Gene and Protein Function

CHS results from mutations in the lysosomal trafficking regulator (*LYST*) gene located on chromosome 1q42.1–42.2, which encodes a large cytoplasmic protein of 3801 amino acids (425 kd) and has unknown function.[68] In some patients, no *LYST* mutations could be identified, suggesting the existence of other CHS-causing genes.[60]

Several proteins interact with LYST, including the SNARE complex protein HRS, signaling protein 14-3-3, and casein kinase II.[69] LYST may act as an adaptor that brings into close proximity proteins that mediate intracellular membrane fusion reactions. LYST contains a beige and Chediak domain (BEACH), a series of WD-40 repeats, ARM motifs, and HEAT repeats.[29] These motifs play roles in vesicle transport and fusion.

GRISCELLI SYNDROME

GS is a rare autosomal recessive disorder characterized by abnormal pigmentation, immunodeficiency, and development of the accelerated phase.[70] GS is caused by mutations in the *RAB27A* gene, a small GTPase involved in vesicular transport and organelle dynamics.[71] GS patients have no obvious bleeding prior to the accelerated phase, and whether they have a defect in platelet dense granules has not been verified. The *ashen* mouse, a model of GS with mutated *RAB27A*, exhibits a reduced number of platelet dense granules,[72] although this finding varied depending on the genetic background.[73] GS patients have hypopigmented hair with a silvery-gray sheen and pigment clumps that are larger and less homogeneously distributed than in CHS.[70] GS cells also lack the giant cytoplasmic granules of CHS. In GS, defective granule exocytosis of T cells results in susceptibility to viral infections and eventually an accelerated phase, similar to CHS.

THROMBOCYTOPENIA ABSENT RADII SYNDROME

TAR is a developmental disorder characterized by thrombocytopenia and bilateral absence of the radii in the presence of thumbs.[74] Platelet counts are extremely low (15 to 30×10^9/L) in infancy, but increase with age and may improve to almost normal by adulthood. The thrombocytopenia becomes symptomatic within the first 4 months of life in more than 90% of cases.[74] Some patients have cardiac and genitourinary involvement. TAR could be either autosomal recessive or dominant.

In one TAR patient, the secondary wave of platelet aggregation in response to ADP and epinephrine was absent, and aggregation in response to collagen was poor.[75]

WISKOTT-ALDRICH SYNDROME

Wiskott-Aldrich syndrome (WAS) is an X-linked recessive disease of microthrombocytopenia, immunodeficiency, and eczema.[76] X-linked thrombocytopenia (XLT) a milder phenotype without immune deficiency, and WAS are both caused by mutations in the *WASP* gene.[77,78] The WASp protein is exclusively expressed in the cytoplasm of hematopoietic cells. It regulates signal-mediated actin cytoskeleton rearrangement by interacting with phosphatidylinositol (4,5)-bisphosphate (PIP2) and Rho-like GTPases (specifically, CDC42) and by stimulating the ARP2/3 actin-nucleating complex.[79]

Bleeding manifestations in WAS range from minor petechiae to serious gastrointestinal or intracranial hemorrhage. Among 154 WAS patients in one study, 30% had life-threatening bleeding before diagnosis.[76] The number of serious bleeding events was similar in all platelet count subgroups, suggesting a qualitative platelet defect.

Several WAS patients have a marked reduction in dense granules, adenine nucleotide storage pools, and platelet aggregation.[80–82] A reduction in α-granules and mitochondria has also been reported in WAS platelets.[81] Patients with XLT also showed a reduced number of dense granules, a-granules and mitochondria.[82] Some carrier mothers have a poor secondary wave of aggregation in response to epinephrine.[80]

CONCLUSION

As occurs repeatedly in medicine, investigation of a disease state leads to elucidation of a normal function that was previously a mystery. For the collection of disorders involving defective δ-granules, that function consists of intracellular vesicle formation from extant membranes. The analysis of this function, however, remains in its infancy, in part because a molecular etiology has been discovered for only a fraction of the pertinent diseases. In the future, our understanding of vesicle formation will emanate from knowledge of the genes and proteins involved in a gamut of δ-storage pool disorders, only a portion of which are currently recognized.

Already, δ-SPD states have taught us a good deal about the cell biology of vesicle formation and trafficking. The HPS diseases tell us that melanosomes in melanocytes and dense bodies in platelets share some elements of their biogenesis. These diseases also reveal that proper intracellular vesicle formation is required for regulation of localized inflammatory responses; when disturbed, the result is granulomatous colitis or pulmonary fibrosis. HPS-2 disease has also emphasized the importance of the β3A subunit of AP3 in the formation of neutrophils via a specific protein, neutrophil elastase.[83] Studies of CHS have uncovered the function of intracellular vesicles in fighting bacterial infections, and investigations into GS indicate the importance of the distal movement of vesicles within cells in controlling T cell activation.[84] With the exception of the role of AP3 in vesicle formation, however, we do not know how the specific molecular defects of δ-SPDs cause their pathology.

Another issue also remains incompletely understood. If HPS genes are ubiquitously expressed, why is the phenotype limited to pigment dilution, bleeding, and sometimes pulmonary and gastrointestinal involvement? One possible explanation is that the formation of melanosomes and dense bodies requires a single, specific pathway, whereas vesicle formation in more generic cells employs redundant mechanisms.

The future remains filled with potential for investigations into δ-SPDs. In all likelihood, subsets of lysosomes will be described based on markers that define them as counterparts of dense bodies or melanosomes, and the behavior of these subsets will be chronicled. Megakaryocytes will be cultured from patients with all varieties of δ-SPD, and the genesis of dense bodies, as well as platelets themselves, will be followed. Immuno-cytochemistry and videoimaging will be applied to the study of normal and mutant megakaryocytes in culture. Given that scores of different proteins work together to accomplish the complex task of new vesicle formation, new diseases involving defects in these proteins will undoubtedly be discovered, and the causative genes will be numbered among those required for vesicle formation in general. Dysfunction will reveal function.

ACKNOWLEDGMENTS

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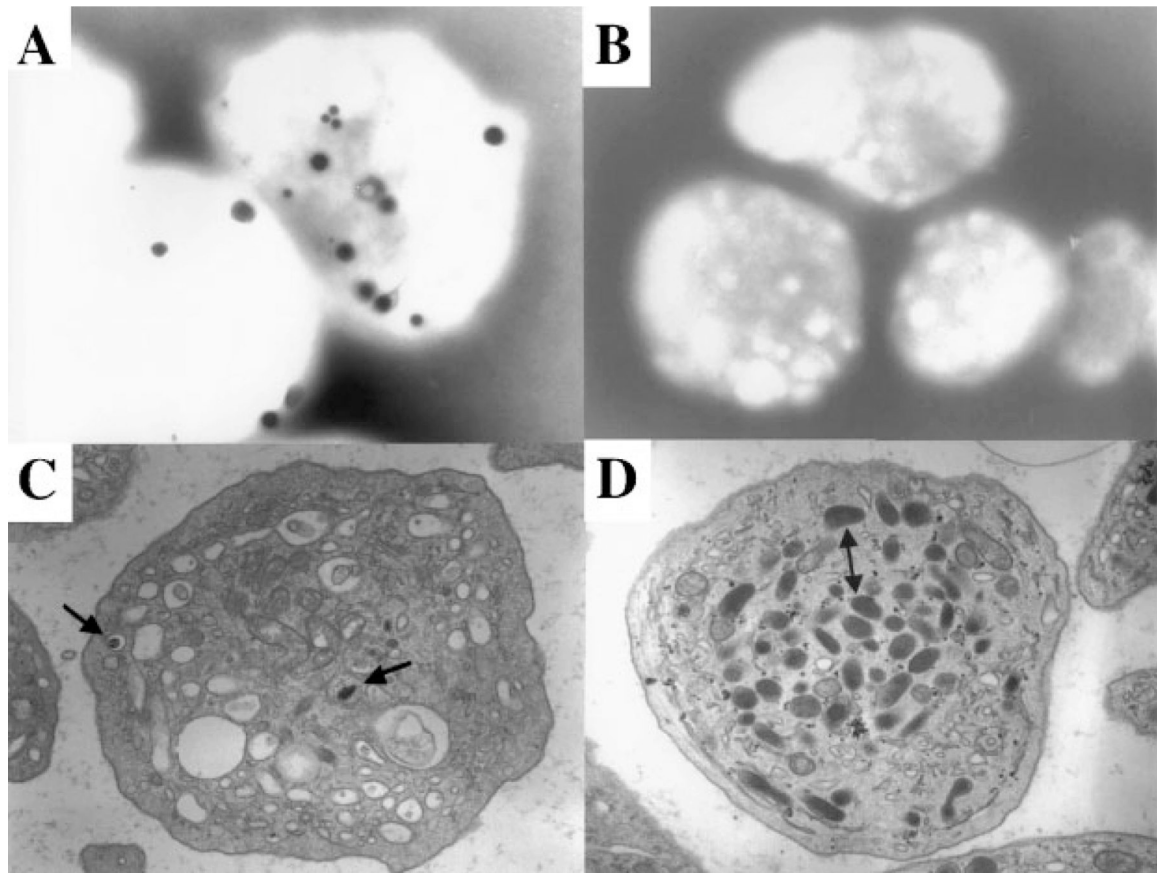84. Trambas CM, Griffiths GM. Delivering the kiss of death. Nat Immunol 2003;4:399–403 [PubMed: 12719728]

Figure 1.
Electron microscopy of platelets showing presence and absence of dense bodies. (A) Whole-mount electron microscopy showing dark, electron-dense granules in the platelets of a 45-year-old woman with albinism but not Hermansky-Pudlak syndrome (HPS). (B) Whole-mount electron microscopy showing absence of dense bodies in the platelets of a 34-year-old man with HPS-1. (C) Transmission electron microscopy showing dense bodies (arrows) in the platelet of a patient with gray platelet syndrome. Note absence of α-granules. (D) Transmission electron microscopy showing absence of dense bodies in a platelet from a 30-year-old woman with HPS-1. Double arrow points to α-granules. (Photographs courtesy of Dr. James G. White, University of Minnesota.)
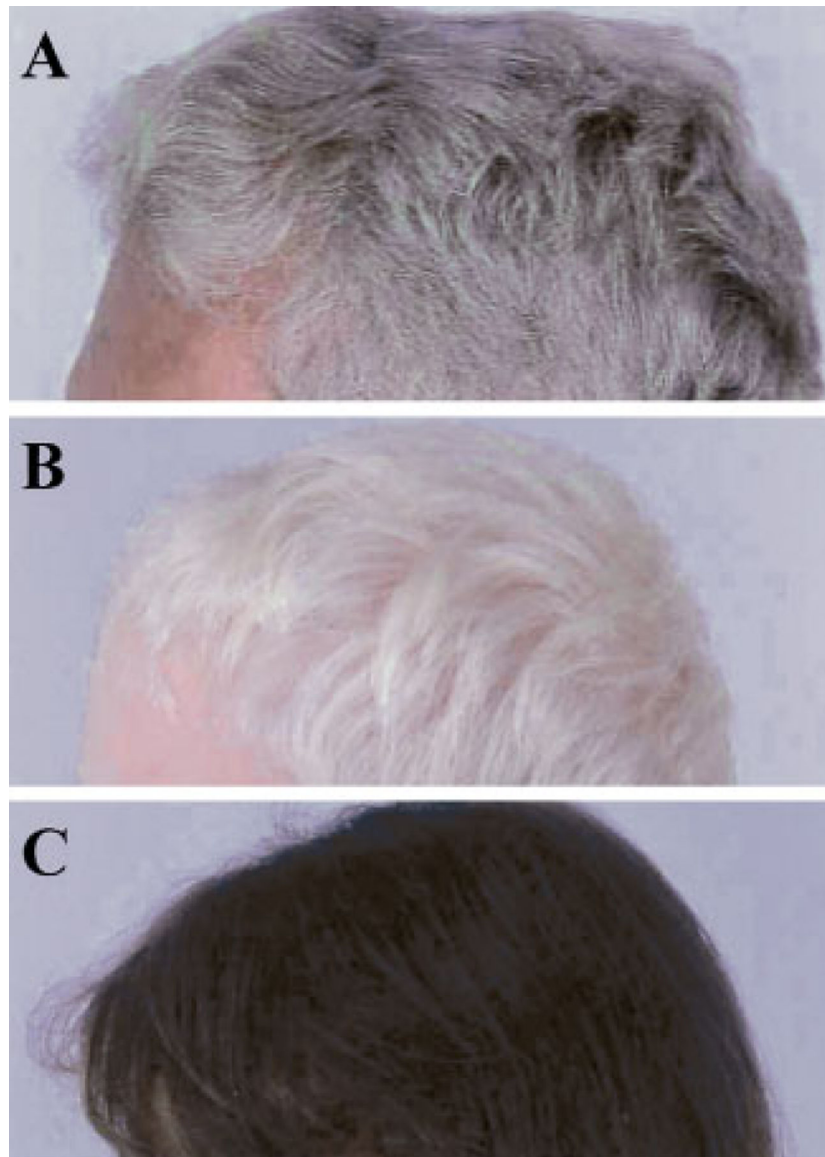
Figure 2.
Hair of three HPS patients, all of Puerto Rican ancestry. (A) Typical gray-brown hair of a 54-year-old man with HPS-1. (B) Completely white hair of a 4-year-old boy with HPS-1. (C) Nearly normal, dark hair of a 5-year-old girl with HPS-3.
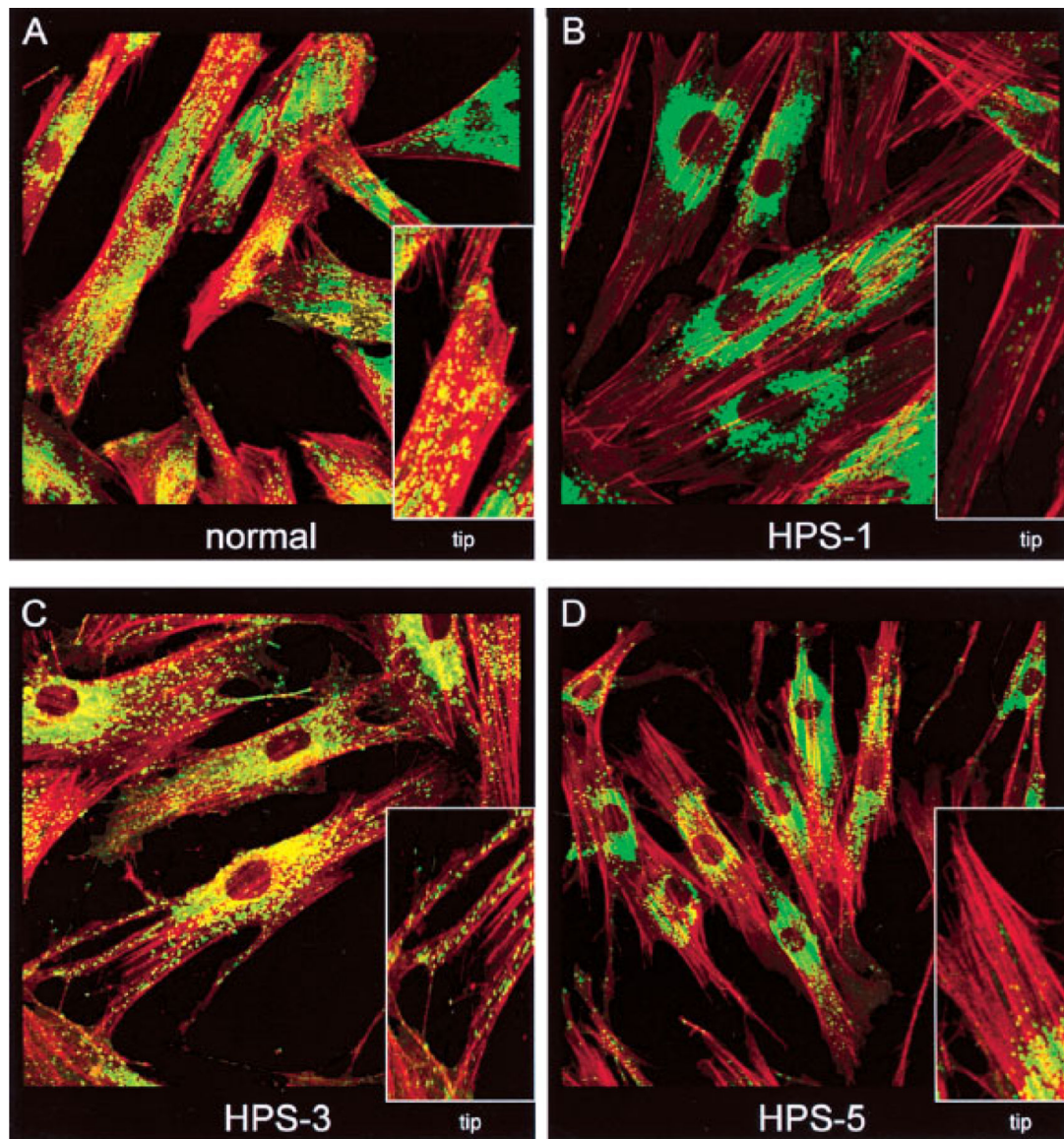
Figure 3.
Intracellular distribution of LAMP3 and F-actin in normal, Hermansky-Pudlak syndrome (HPS)-1, HPS-3, and HPS-5 fibroblasts. Fibroblasts were fixed on coverslips and stained with mouse monoclonal antibodies to LAMP3 (as described in Huizing et al[41]) and F-actin (Phalloidin, Molecular Probes, Eugene, OR). F-actin staining (red) was employed to mark the outline of the cells, in particular the dendritic tips. Insets show isolated dendritic tips. (A) Normal fibroblasts show a punctate LAMP3 (green) distribution throughout the cell, including at the ends of the dendritic tips. (B) HPS-1 fibroblasts show a clumped accumulation of LAMP3 in the perinuclear area. (C) HPS-3 fibroblasts appear to have a nearly normal LAMP3 distribution, with LAMP3 reaching into the dendritic tips. (D) HPS-5 cells have minimal LAMP3 transport into the tips, but LAMP3 does not accumulate in clumps in the perinuclear area, as in HPS-1. (Photographs produced by Heidi Dorward.)

Table 1

Vital Information about HPS Genes

| HPS Subtype | Gene | Chromosome | Size | | | Mouse | No. of Patients | Comments |
			gDNA (kb)	mRNA (kb)[*]	Exons	Protein (kd)[*]			
1	HPS-1	10q23.1–13.3	31.5	3.7	20	79.3	pale ear	450–500	NW PR isolate
2	AP3B1	5q14.1	292.3	4.2	27	140	pearl	4	
3	HPS-3	3q24	43.9	4.4	17	113.7	cocoa	23	Central PR isolate
4	HPS-4	22cen-q12.3	32.3	4.5	14	76.9	light ear	14	Interacts with HPS-1
5	HPS-5	11p14	43.4	4.8	23	127.4	ruby eye-2	3	
6	HPS-6	10q24.32	2.7	2.7	1	83.0	ruby eye	3	
7	HPS-7	6p22.3	140.2	1.5	10	39.5	sandy	1	

[*]
mRNA and protein size of the longest transcript forms.

HPS, Hermansky-Pudlak syndrome; PR, Puerto Rico.