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To the editor:

Exome sequencing reveals a pallidin mutation in a Hermansky-Pudlak–like primary immunodeficiency syndrome

Partial albinism and primary immunodeficiency occur in several autosomal recessive disorders, including Hermansky-Pudlak syndrome type 2 (HPS2, Online Mendelian Inheritance in Man [MIM] #608233), Chediak-Higashi syndrome (MIM#214500), Griscelli syndrome types 1 (MIM#214450) and 2 (MIM#607624), and endosomal-adaptor protein p14 deficiency (MIM#610798).[1-6] At least 15 recessive mouse mutations have been described that also are characterized by partial albinism and immunodeficiency and/or bleeding disorders and that appear to be homologous to the human diseases.[7-10]

A 17-year-old, northern Italian female with oculocutaneous albinism, nystagmus, and normal neurologic development presented with recurrent cutaneous infections but without hemorrhagic episodes. At 6 years of age, she had a prolonged episode of fever with convulsions. At presentation, she had thrombocytopenia (111 000 platelets/μL) and leucopenia (2600 leucocytes/μL, 2300 neutrophils/μL; 300 lymphocytes/μL). Platelet aggregation tests were normal.

Nucleotides (37.7 million) of exons (the exome) were enriched 44-fold from genomic DNA from the patient and sequenced to an average, uniquely aligned coverage of 135-fold.[11] No mutations were identified in known immunodeficiency disease genes. Only one novel variant had high likelihood of pathogenicity and was unique to the patient among ~ 250 Children's Mercy Hospital exomes and the NHLBI exome collection (http://evs.gs.washington.edu/EVS/). It was a homozygous nonsense mutation, c.232C > T (p.Q78X), in exon 3 of pallidin (*PLDN*, chr15:45895305C > T, relative to human genome build 37, supplemental Figure 1A [available on the *Blood* Web site; see the Supplemental Materials link at the top of the online article]), present in all 65 aligning sequences. This mutation was confirmed to be homozygous in the patient and heterozygous in her parents by Sanger sequencing (supplemental Figure 1B), and was associated with absent PLDN protein expression (supplemental Figure 1C).

Because intracellular trafficking and degranulation of specialized lysosomes are impaired in HPS2,[2,3,5] we sought such defects in the patient. The proportion of resting and IL-2–activated NK cells expressing the lysosomal membrane protein CD107a on the surface was increased (6% and 14% of NK cells from the patient, respectively, versus 0.6% and 2% in healthy controls, respectively, Figure 1A). However, these increases were not as marked as in HPS2 (24% in IL-2–activated NK cells [S.P., G.T., R.B., unpublished observations] but at odds with one case report[4,5]). PLDN replacement in NK cells from the patient decreased CD107a expression to normal (Figure 1B).

NK cells from the patient had intermediate cell-surface expression of CD63, another lysosomal membrane protein altered in HPS2[1,4,6] (17% in patient, 9% in controls, and 28% in an HPS2 patient, Figure 1A). Degranulation, as measured by change in surface expression of CD107a on IL-2–activated NK cells after challenge with LCL 721.221 target cells, was less than controls (Figure 1C).

Cytolytic activity of resting and IL-2–activated NK cells from the patient was reduced (Figure 1C). Low NK activity did not correlate with reduced expression of activating receptors,[12] as assessed by NKp30, NKp46, NKG2D, and CD244 (2B4) levels on NK cells cultured with IL-2 for 3 weeks.

Pallid mice have reductions in coat and eye pigmentation, lysosomal enzyme secretion, chemotactic release of neutrophil elastase, and neutrophil killing of *Leishmania,* together with prolonged bleeding because of storage pool deficiency.[7-10] Here, we

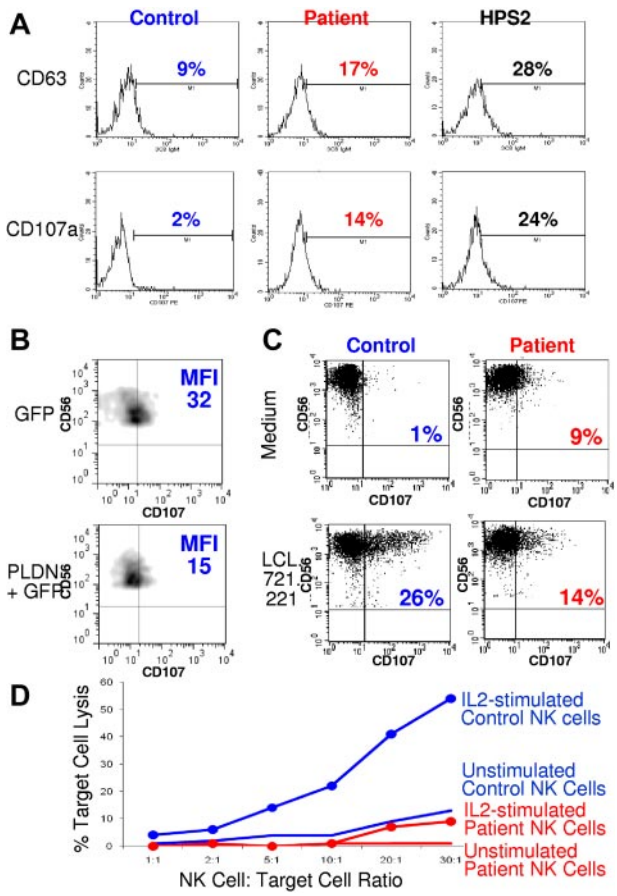

Figure 1. Effects of a *PLDN* mutation on NK-cell function. (A) Histograms of flow cytometric measurement of CD63 and CD107a on IL-2–activated NK cells from a normal control subject, the pallidin-deficient patient, and a patient with HPS2. (B) Two-color flow cytometric measurement of CD107a and CD56 on IL-2–activated NK cells from the pallidin-deficient patient, after transfection with expression vectors containing GFP or PLDN and GFP. (C) Two-color flow cytometric measurement of CD107a on IL-2 activated NK cells from a normal control subject and the pallidin-deficient patient, after culture with medium or with target cell line LCL.721.221. (D) Lysis of K562 NK target cells by freshly isolated PBMCs from the pallidin-deficient patient and a healthy control before and after overnight incubation with IL-2. Experiments were repeated 3 times in 3 independent experiments.

have shown that a homozygous nonsense mutation in the homologous human gene was associated with partial oculocutaneous albinism, leucopenia, and recurrent infections. While in preparation for publication, a patient with partial albinism and absence of platelet δ granules was reported to have the same mutation in *PLDN*.[13] That patient did not have recurrent infections. We have also shown the *PLDN* mutation to be associated with defective NK-cell degranulation and cytolysis, and with abnormal lysosomal markers on NK cells that were similar to, but distinct from, those in HPS2. Some of these were corrected by PLDN replacement. The symbol HPS9 has been approved for PLDN-associated disease.

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Contribution: R.B. obtained the clinical information, led the functional studies and wrote the manuscript; S.C. and F.C. performed Sanger sequencing and immunoblotting studies; M.E.C. and A.P. were following the child; A.P. has performed PLDN sequence analysis in control subjects and immunoblotting experiments; M.G. has performed flow cytometry and neutrophil studies; G.T. and S.P. have performed NK cell studies; C.J.B. contributed computer programming and data analysis; D.L.D. made the sequencing libraries, performed target enrichments and data analysis; A.P. performed the transfection studies; N.A.M. carried out data pipelining, software development and bioinformatics; S.L.H. carried out literature research and data analysis; L.Z. and G.P.S. performed sequencing; and S.F.K. wrote the manuscript and carried out data analysis.

Conflict-of-interest disclosure: L.Z. and G.P.S. were employees of Illumina Inc at the time the research was performed. The remaining authors declare no competing financial interests.

The PLDN mutation was deposited at the NCBI ClinVar.

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To the editor:

Decreased hepcidin expression in murine β-thalassemia is associated with suppression of Bmp/Smad signaling

β-thalassemia is a genetic disorder of hemoglobin production characterized by ineffective erythropoiesis and anemia.[1] Iron overload, a major source of morbidity, results from inappropriately low expression of the gene encoding hepcidin (*Hamp1*).[1] Hamp1 controls plasma iron concentration by facilitating the degradation of the iron efflux protein ferroportin.[2] In healthy individuals, hepcidin istranscriptionally responsive to iron via bone morphogenetic protein (Bmp)/Smad pathway-mediated phosphorylation of Smad 1,5,8.[3] Phosphorylated Smad 1,5,8 is an essential component of the transcriptional complex that induces *Hamp1* expression in response to iron.[4] We investigated the

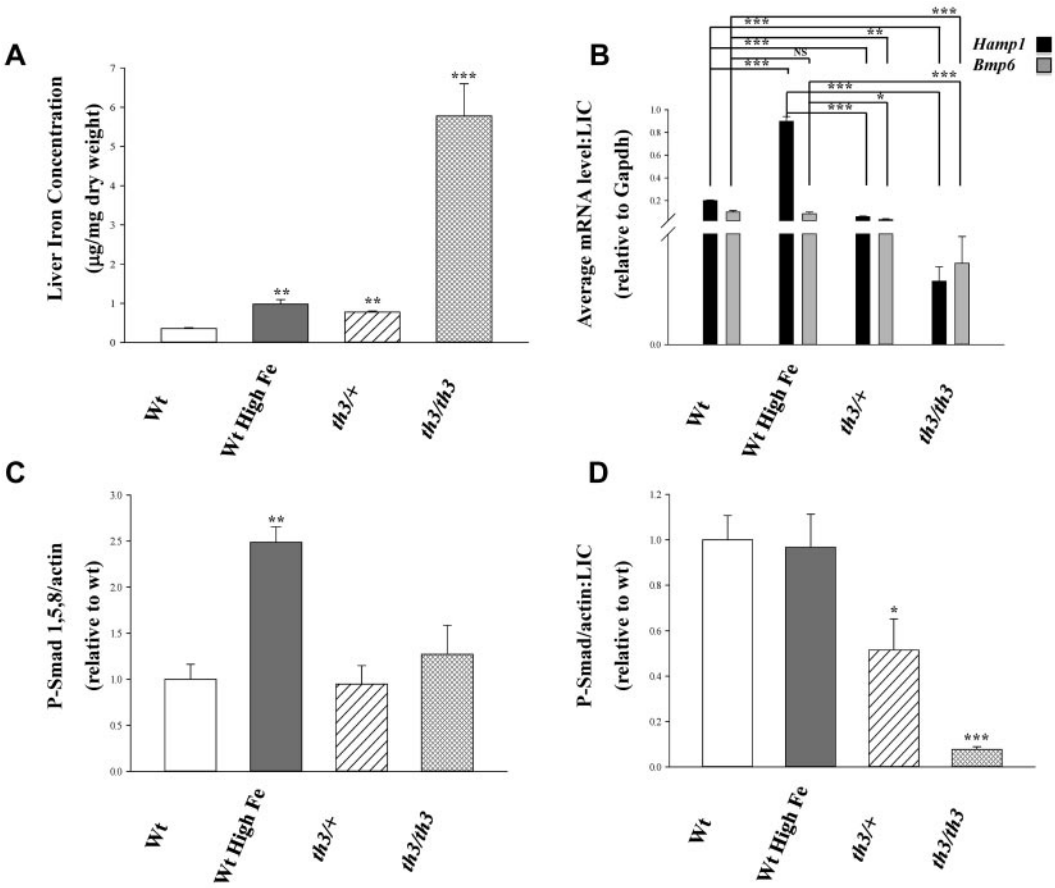

Figure 1. Bmp/Smad signaling is hyporesponsive to iron in β-thalassemic mice. (A) Liver iron concentrations (μg/mg dry weight) from wt mice fed a standard diet (200 ppm iron), wt mice fed a high iron diet (standard chow supplemented with 2.5% carbonyl iron), *th3*/+, and *th3/th3* mice were determined by atomic absorption and are expressed as mean ± SEM. (B) Average fold difference in the transcription levels of the Bmp/Smad pathway member *Bmp6* and target gene *Hamp1* were normalized to LIC. Data were normalized to mouse Gapdh and are presented as the mean ± SEM ($n \geq 3$). All averaged values are the product of duplicate determinations. (C) Chemiluminesence signals from Western blots of liver lysates reacted with antibodies to phosphorylated Smad 1,5,8 and to β-actin were quantified and data expressed as the mean ± SEM of their ratios. (D) Phosphorylated Smad 1,5,8 levels normalized to β-actin are expressed relative to LIC. Data are presented as mean ± SEM. Statistical significance was determined using the Student *t* test. $^*P \leq .05$, $^{**}P \leq .01$, $^{***}P \leq .001$. Significance with respect to wt or iron-loaded wt values are indicated by the brackets.