

Letter to the Editor

A novel Rab27a mutation binds melanophilin, but not Munc13-4, causing immunodeficiency without albinism

To the Editor:

The small GTPase Rab27a regulates a number of exocytic processes. In melanocytes Rab27a interacts with melanophilin to mediate the melanosome transport required for pigmentation.¹ In natural killer (NK) cells and cytotoxic T lymphocytes, degranulation and thereby cell-mediated cytotoxicity require interaction of the active form of Rab27a with Munc13-4.^{2,3} Mutations in the human *RAB27A* gene cause the autosomal recessive Griscelli syndrome type 2 (GS2) with diluted pigmentation and immunodeficiency, which can progress to hemophagocytic lymphohistiocytosis (HLH).⁴ Recently, 6 patients with GS2 and normal pigmentation have been reported, identifying Rab27a mutations that selectively disrupt Munc13-4 binding when re-expressed in HEK293 cells.⁵ Here we present a new case of GS2 with no dilution of pigmentation and extend prior biochemical results through physiologic cell expression studies.

Death from HLH of 2 children of a first-cousin consanguineous union led us to investigate the genetic background of the family (for clinical details, see the [Methods](#) section in this article's Online Repository at www.jacionline.org). Targeted sequencing for the known HLH genes was unrevealing. Although pigmentation was normal, whole-exome sequencing identified a novel *RAB27A* mutation at c.244C>T (p.R82C), which was confirmed by means of Sanger sequencing and suggested the diagnosis of GS2. One of 2 deceased children for whom DNA was available (II.2), as well as 2 living siblings (II.4 and II.5), were homozygous for c.244C>T. The other 3 living children (II.3, II.6, and II.7) were heterozygous for the mutation ([Fig 1, A](#)). All 5 living siblings had unremarkable courses before and at the time of this report.

The atypical phenotype for GS2 prompted us to investigate the patients' mutations further. Blood and hair samples were obtained with informed consent in compliance with the guidelines of the Institutional Review Boards of the Children's Hospital of Philadelphia and Baylor College of Medicine. A patient with GS2 with diluted pigmentation and progression to HLH (classical GS2) who had a homozygous *RAB27A* mutation (c.220G>C p.D74H) was included in this study (clinical details of this case will be reported elsewhere). We examined all patients' hair by using differential interference contrast microscopy. The hair pigment of the classical patient with GS2 was distributed irregularly in large clumps ([Fig 1, B, D74H](#)). For all 5 homozygous and heterozygous R82C patients, hair pigmentation was more uniform with rare small clumps and not consistent with typical hair of patients with GS2 ([Fig 1, B](#)). We studied the NK cell function of 4 of the 5 living patients (II.3, II.4, II.5, and II.6) in detail to determine the effect of the Rab27a mutation on NK cell cytotoxicity. NK cell numbers in patients were within low to normal ranges (see the [Methods](#) section in this article's Online Repository). Standard 4-hour ⁵¹Cr release assays (performed as described previously)⁶ of PBMCs against the human erythromyeloblastoid leukemia cell line K562 demonstrated that the NK cell-mediated cytotoxicity was reduced in all 4

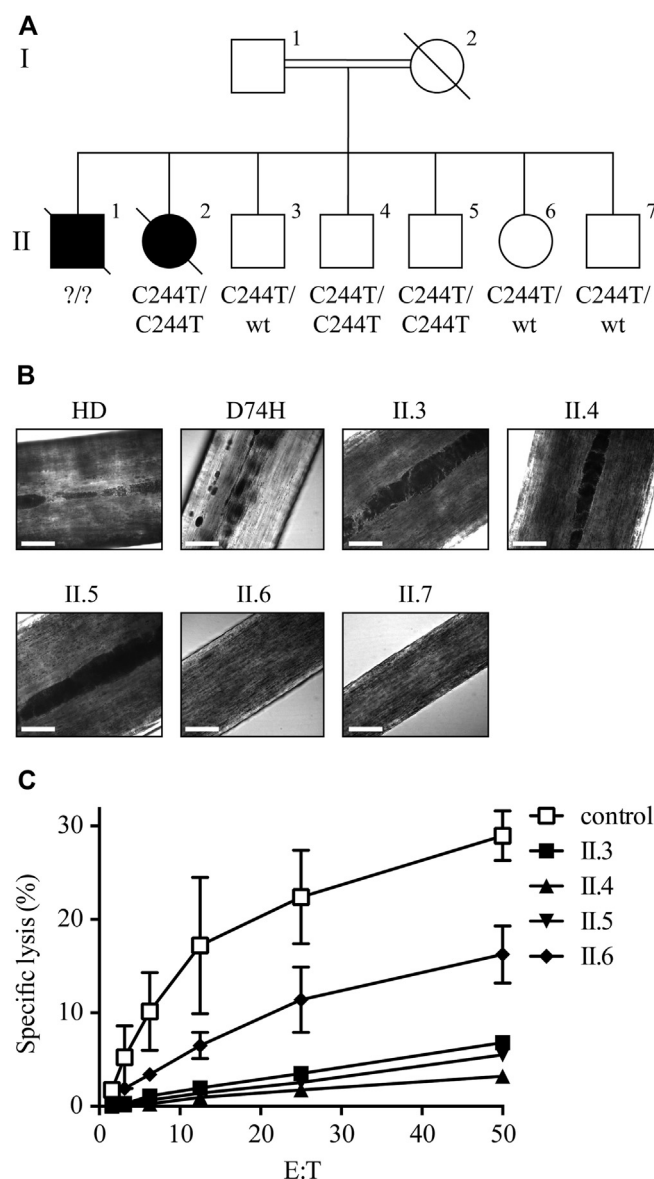


FIG 1. Rab27a R82C does not alter hair pigmentation but decreases NK cell-mediated cytotoxicity. **A**, Family pedigree. Parents are first-degree cousins. Patients II.1 and II.2 died. wt, Wild-type. **B**, Differential interference contrast images of hair from patients and a healthy donor (HD; $\times 63$ magnification). Scale bar = 25 μ m. **C**, NK cell cytotoxicity in PBMCs from patients and a healthy control subject measured in a standard 4-hour ⁵¹chromium release assay against K562 target cells (n = 2). E:T, Effector cell/target cell ratio.

siblings ([Fig 1, C](#)). Both homozygous patients (II.4 and II.5) have normal NK cell numbers but reduced CD107a mobilization, which could explain the severely decreased cytotoxic NK cell function. The heterozygous siblings (II.3 and II.6) are less affected. The different cytotoxic responses observed for II.3 and II.6 could be caused by different percentages of NK cells detected for these patients (see the [Methods](#) section in this article's Online Repository). In all 4 patients tested, the NK cell-mediated cytotoxicity could be rescued only partially by IL-2 treatment (see [Fig E1](#) in this article's Online Repository at www.jacionline.org).

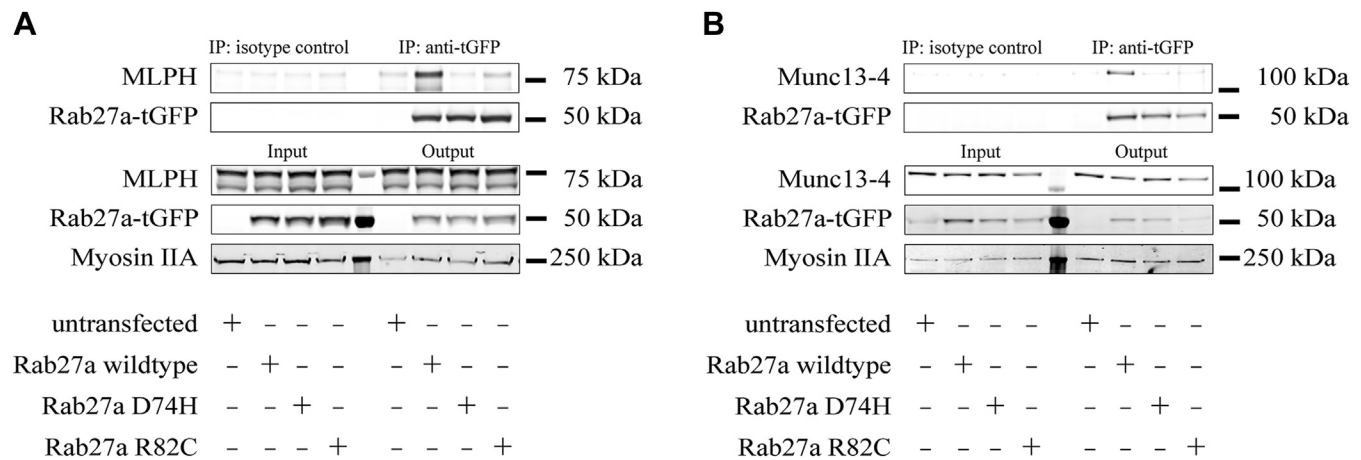


FIG 2. Rab27a mutation at R82C selectively disrupts binding to Munc13-4 but not melanophilin (MLPH). tGFP-tagged wild-type Rab27a and Rab27a D74H and R82C mutations were expressed in mel1106 (A) and NK92 (B) cells. Cells were lysed in 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate buffer, and lysates were incubated first with isotype control-coated beads (clone MPC-11) and subsequently with anti-tGFP-coated beads (clone 2H8). Whole-cell lysates and precipitates were separated in SDS-PAGE (4-12%), proteins transferred onto nitrocellulose membrane, and membrane probed with anti-Rab27a (clone 1G7) and anti-MLPH (Fig 2, A) or anti-Munc13-4 (Fig 2, B) antibodies (n = 3).

Impaired cytotoxicity without diluted pigmentation in the patients led to the hypothesis that the mutation in *RAB27A* at c.244C>T (p.R82C) differentially affects the downstream function of the protein in NK cells compared with pigment-producing cells. More specifically, this novel mutation would disrupt Rab27a binding to Munc13-4 but not affect interaction with melanophilin, leading to impaired cytotoxic response with normal pigmentation, as observed in the patients.

To test this hypothesis in physiologically relevant settings, we overexpressed Turbo-green fluorescent protein (tGFP)-tagged wild-type Rab27a or Rab27a R82C or D74H mutations in the NK cell line NK92 and the melanoma cell line mel1106. The Rab27a variants were immunoprecipitated by using anti-tGFP-coated magnetic beads, and coimmunoprecipitation of melanophilin (mel1106) or Munc13-4 (NK92) was detected by means of immunoblotting. Rab27a R82C bound melanophilin, although to a lesser extent than wild-type Rab27a (Fig 2, A). In contrast, no Munc13-4 was detectable in the Rab27a R82C precipitate (Fig 2, B). Neither melanophilin nor Munc13-4 coimmunoprecipitated with Rab27a D74H (classical GS2; Fig 2). Quantifying 3 independent experiments, we detected that Rab27a R82C coprecipitated 2.7 ± 1.4 -fold more melanophilin than D74H. The amount of Munc13-4 in the precipitate was comparable between the 2 Rab27a mutants (1.2 ± 1 -fold). The results supported the conclusion that the mutation at R82C inhibits interaction of Rab27a with Munc13-4 but only partially affects binding of Rab27a to melanophilin and that this selective effect causes atypical GS2 with normal pigmentation.

Our data are consistent with a recent report of patients with biallelic *RAB27A* mutations and normal pigmentation with absent or reduced degranulation and cytotoxicity.⁵ Those authors identified Rab27a mutations that reduced binding to Munc13-4 but did not affect binding to melanophilin in HEK293 cells. Here we report another homozygous Rab27a mutation (R82C) that selectively binds melanophilin in melanocytes but not Munc13-4 in NK cells. Using NK and melanoma cell lines, we have investigated the binding activity of the mutations to the

endogenously expressed interaction partners in comparison with wild-type Rab27a. Thus we confirm the previously described biology in a truly physiologic context using cells that have full ability to mediate the functions of cytotoxicity or pigmentation. Our data indicate that the absent binding of Rab27a R82C to Munc13-4 in NK cells causes the cytotoxic deficiency observed in the patient's PBMCs and that the residual binding activity of R82C to melanophilin is sufficient to allow normal pigmentation in melanocytes.

Overall, our study underscores the importance of unbiased genetic sequencing paired with biological and functional analysis to determine causes of atypical presentation of immune deficiency to allow for the most appropriate and timely treatment of patients. Exceptions to canonical phenotypes of primary immunodeficiency are becoming increasingly common because of increasing access to genomic diagnosis. The ability to pursue the biology of these extended phenotypes is essential to provide the proof to advance basic immunology, as well as the diagnoses needed to advance clinical care with confidence.

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METHODS

Case 1: II.2 (C244T/C244T)

A 9-year-old girl born to a first-cousin consanguineous union had an otherwise unremarkable medical and developmental history other than having received a routine course of isoniazid treatment at age 3 years after having contact with an active case of tuberculosis.

At age 9 years, she presented with intermittent fever treated with oral antibiotics for suspected bacterial infections. One week later, she presented with 2 days of fever, ataxic gait, and slurred speech. She had upward and lateral gaze nystagmus, generalized hypotonia, bilateral tremors, and head titubation with intact cranial nerves. She required intensive care and mechanically assisted ventilation. Peripheral hematologic counts were as follows: normal hemoglobin level, WBC of 3700 cells/ μ L (absolute neutrophil count [ANC], 1665 cells/ μ L; absolute lymphocyte count [ALC], 1998 cells/ μ L), and 350,000/ μ L platelets. Except for increased protein levels (600 mg/L), cerebrospinal fluid (CSF) was normal and negative for bacteria, mycobacteria, and fungi. Serum and CSF were evaluated by PCR and were negative for cytomegalovirus (CMV), herpes simplex virus, enteroviruses, and varicella virus. Head computed tomographic scans were normal, and she was treated with 1 dose of intravenous immunoglobulin for suspected Guillain-Barré syndrome and 10 days of intravenous ceftriaxone, azithromycin, and acyclovir. Fever persisted (40°C), and she was given a diagnosis of pneumonia.

One week later, she had worsening ataxia and speech with profound right-sided hypotonia, loss of consciousness, loss of gag and abdominal reflexes, and dilated but reactive pupils, and she required assisted ventilation. Her hemoglobin level was 10.6 g/dL, WBC was 1,650 cells/ μ L (ANC, 501 cells/ μ L; ALC, 890 cells/ μ L), and platelets were 131,000/ μ L. Serum ferritin and triglyceride levels were normal. Head magnetic resonance imaging (MRI) showed meningoencephalitis with predominant posterior fossa involvement. Bone marrow aspirate revealed hypocellularity and only evidence of reactivity without other abnormality. Her immunoglobulin levels were normal, with the exception of increased IgE levels (440 kU/L). Analysis of CSF demonstrated increased protein levels (1.08 g/L) without cells and no bacterial growth. Lymphocyte subset analysis was remarkable for lymphopenia (ALC, 880 cells/ μ L), with 95% CD3⁺ T cells (863 cells/ μ L), 52% CD4⁺ T cells (458 cells/ μ L), 40% CD8⁺ T cells (352 cells/ μ L), 2% CD19⁺ B cells (176 cells/ μ L), and 0.7% CD56⁺ NK cells (62 cells/ μ L). She had pulmonary hemorrhage, and ganciclovir treatment was started because of CMV having been identified in her bronchoalveolar lavage specimen.

Four months later, head MRI was repeated and demonstrated generalized large lesions in the right frontal lobe, as well as extensive low-attenuation changes within the midbrain. An open lung biopsy was performed, and histologic examination identified only lymphocytic interstitial pneumonia. She was presumed to have and was treated for HLH with high-dose dexamethasone, antithymocyte globulin, mycophenolate mofetil, and alemtuzumab (anti-CD25 mAb). She was weaned from ventilator support but had bulbar palsy, left-sided hemiplegia, and ataxia and required a tracheotomy with ongoing mechanical ventilatory assistance. Several months thereafter, she developed septic shock and died of acute respiratory distress syndrome. Sanger sequencing for *RAB27A* detected that the patient was homozygous for c.244C>T (p.R82C), which retrospectively confirmed the diagnosis of HLH.

Case 2: II.1 (??/?)

A 10-year-old boy was previously generally healthy and, like his sister (case 1), treated with isoniazid prophylaxis for exposure to active tuberculosis. He had the additional medical history at age 6 years of needing treatment for brucellosis (*Brucella melitensis* titer, 1:5120) with 3 weeks of rifampicin and trimethoprim-sulfamethoxazole.

At age 10 years, he had fever with a hemoglobin level of 8.9 g/dL, WBC was 2,400 cells/ μ L (ANC, 800 cells/ μ L; ALC, 1,300 cells/ μ L), platelet count was 121,000/ μ L, and serum ferritin level of 81 μ g/L. One week later, he had hepatosplenomegaly, with normal liver function test results. After 3 months, he had mild jaundice with a serum unconjugated bilirubin level of 60 μ mol/L and a conjugated bilirubin levels of less than 2.0. Serum PCR results were negative for adenovirus, CMV, and EBV. The serum IgG level was 1200 mg/

dL, IgA level was 147 mg/dL, and IgM level was 107 mg/dL. He was treated with oral prednisolone, cyclosporine, trimethoprim-sulfamethoxazole prophylaxis, and intravenous immunoglobulin. The hepatosplenomegaly improved thereafter with normalizing cell counts (except for a platelet count of 121,000 cells/ μ L).

At 11 years of age, he had headache, diplopia, and muscle weakness, with preference for the lower extremities. Head MRI demonstrated hemorrhagic lesions in the frontal and nonhemorrhagic lesions in the right mesial lobes. Serum virologic studies detected EBV DNA (1,779 copies/mL). Results of CSF cultures, including those for acid-fast bacilli, as well as PCR for enteroviruses, John Cunningham virus, BK virus, and parvovirus B19, were all negative. Results of skin biopsy for him and for his sister were the same, revealing monoclonal T-cell populations (mixed CD4 and CD8 cells). Over 4 weeks, he deteriorated, with progressive pancytopenia, multiple intracranial hemorrhagic lesions and intractable seizures, demyelinating sensorimotor neuropathy, and other infections. He did not respond to intensive care-level treatment, including mechanically assisted ventilation, intravenous antimicrobials, daclizumab (anti-CD25 mAb), and alemtuzumab, high-dose dexamethasone, and he subsequently died.

Case 3: II.3 (C244T/wild-type)

The patient is male and has been evaluated for familial HLH syndromes since childhood because of his family history. He had an unremarkable course. Repeated CBC, WBC with differential, platelet counts, liver function tests, and kidney function tests were normal. Serum IgG, IgA, IgM, and IgG subclasses; anti-vaccine antibody titers; and lymphocyte subsets were normal, except for low CD56. PCR results for CMV, EBV, and adenovirus were negative. Between 11 and 16 years of age, he had low to borderline normal NK cell numbers (4% to 5% of lymphocytes) and decreased to absent NK cell function (average, 0.1 lytic units [LU], $n = 7$). CD107a mobilization was normal (15%; mean channel fluorescence [MCF], 165 [range, 10% to 37%; MCF, 152-986]).

Case 4: II.4 (C244T/C244T)

The patient is male and has been evaluated for familial HLH syndromes since the age of 8 years because of his family history. He had an unremarkable course except for persistence of low EBV viremia (64-562 copies/ μ g) for 5 years. PCR results for CMV and adenovirus were negative. Between 7 and 12 years of age, his NK cell numbers were normal (7% to 15% of lymphocytes), with decreased to absent NK cell function (average LU, <0.3; $n = 7$). CD107a mobilization upon activation was low (10%; MCF, 114 [range, 10% to 37%; MCF, 152-986]).

Case 5: II.5 (C244T/C244T)

The patient is male and has been evaluated for familial HLH syndromes since the age of 5 years because of his family history. He had an unremarkable course. Between 6 and 10 years of age, he had EBV viremia (80-932 copies/ μ g), but PCR results for CMV and adenovirus were negative. Results of CMV serology was positive, documenting past infection. His lymphocyte subsets were essentially normal. Between 5 and 10 years of age, his NK cell numbers were 6% to 17% of lymphocytes, but NK cell function was decreased to absent (average LU, 0.09; $n = 5$). CD107a mobilization on activation was decreased (6%; MCF, 75 [range, 10% to 37%; MCF, 152-986]).

Case 6: II.6 (C244T/wild-type)

The patient is female and has been evaluated for familial HLH syndromes since the age of 5 years because of her family history. She had an unremarkable course. Repeated CBC, WBC with differential, platelet counts, liver function tests, and kidney function tests were normal. Serum IgG, IgA, IgM, and IgG subclasses; anti-vaccine antibody titers; and lymphocyte subsets were normal. Between 2 and 5 years of age, she had low EBV viremia (80-219 copies/ μ g), which has subsequently returned to undetectable levels. Results of quantitative studies for CMV and adenovirus were negative. Between 1 and 5 years of age,

her NK cell numbers were normal (5% to 11% of lymphocytes), and NK cell function was absent to borderline normal (average, 0.9 LU; n = 3). CD107a mobilization on activation was normal (14%; MCF, 206 [range, 10% to 37%; MCF, 152-986]).

Case 7: II.7 (C244T/wild-type)

The patient is male and has been evaluated for familial HLH syndromes since birth because of his family history. He had an unremarkable course. Immunologic studies were normal, as were quantitative tests for adenovirus,

CMV, and EBV in the peripheral blood. At 2 years of age, NK cell frequency was 13% of lymphocytes, but NK cell function was decreased to absent (average, 1.0 LU; n = 2) with decreased CD107a mobilization on activation (9%; MCF, 149 [range, 11% to 35%; MCF, 207-678]).

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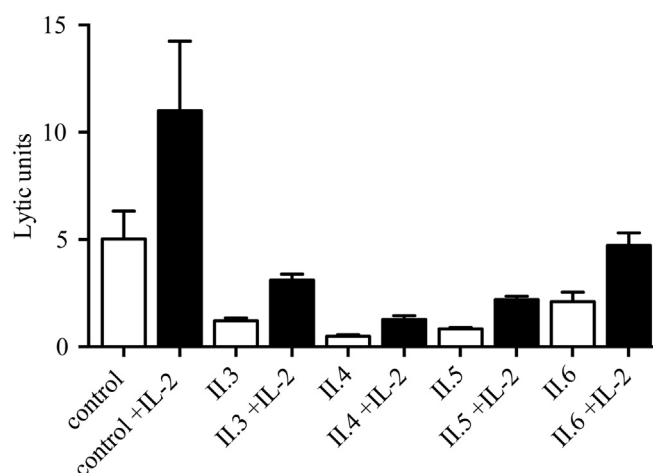


FIG E1. NK cell-mediated cytotoxicity is partially rescued by IL-2. Lytic units, defined as the number of NK cells $\times 10^7$ required to mediate 20% of target cell lysis, were used as a measurement of cytolytic capacity and were determined as described previously.^{E1} PBMCs from a healthy control subject and the patients were either untreated (*white bars*) or incubated with IL-2 30 minutes before the standard 4-hour ⁵¹chromium release assay against K562 target cells (*black bars*; n = 2).