

# Griscelli Syndrome: Rare Neonatal Syndrome of Recurrent Hemophagocytosis

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**Abstract:** Griscelli syndrome (GS) is a rare inherited disease characterized by immunodeficiency and partial albinism. The microscopic findings of the skin and hair are highly suggestive of the disease. The GS locus colocalizes on chromosome 15q21 with the *myosin-Va* gene (*MYO5a*), and mutations have been identified in few patients. We describe a 2-month-old Hispanic girl with severe pancytopenia secondary to hemophagocytosis. Even though a mutation at the Griscelli locus had not been identified, her clinical features and outcome were typical of GS. The purpose of this article is to alert physicians to the association between GS and hemophagocytosis. We suggest that GS should be considered in infants with hemophagocytosis because the features of partial albinism can be subtle. The relevant literature is summarized.

**Key Words:** Griscelli—Hemophagocytosis—Immune deficiency—Neonate—Pancytopenia.

Griscelli syndrome (GS) was first described in 1978 by Griscelli et al. (1) in two patients and Siccardi et al. (2) in one patient. Since then, there have been about 30 cases described in the literature. The main features of GS are partial pigmentary dilution with silver-gray hair, variable cellular immune deficiency including decreased T-cell and natural killer (NK) cell cytotoxicity, and recurrent acute phases of uncontrolled lymphocyte and macrophage activation resulting in hemophagocytosis in different organs (1,3). Most cases described are in the Turkish and Mediterranean population. The mean age at diagnosis is variable, and both genders are affected equally. All patients have a silver-gray sheen to their hair, but the skin may have varying degrees of hypopigmentation. By a mean age of 36 months, hemophagocytosis occurs, perhaps secondary to a viral insult; patients have fever, hepatosplenomegaly, central nervous system (CNS) infiltration by the activated lymphocytes and macrophages, coagulopathy and pancytopenia (3). The outcome is generally fatal, even with chemotherapy and immunosuppressive treatments. The mean age of death is 5 years from progressive CNS disease or recurrent infections. Some children are alive after successful bone marrow trans-

plantation. The light microscopic and electron microscopic findings of hair and skin are characteristic. The Griscelli syndrome locus has recently been shown to colocalize at 15q21 with the *myosin-Va* gene, *MYO5a* (4). This is an unconventional myosin with a proposed role in membrane transport and organelle trafficking inside cells. It is therefore postulated that in Griscelli syndrome, impairment of this process results in pigmentary changes and altered lymphocyte function resulting in immune deficiency (5).

There are two main syndromes of tyrosinase-positive pigmentary dilution (albinism) that have to be differentiated from GS: Chediak-Higashi syndrome and Hermansky-Pudlak syndrome. The presence of characteristic large granules in neutrophils and defective chemotaxis and bactericidal activities of the neutrophils differentiate the prototype disorder of Chediak-Higashi syndrome. In the skin, the pigmentary dilution is present in both the melanocytes and keratinocytes, and on electron microscopy, giant melanosomes are seen. The pigment in the hair shafts is granular and more evenly distributed. Hermansky-Pudlak syndrome, the other pigmentary disorder, has a significant platelet function defect and a lifelong bleeding tendency associated with it; however, there is no impairment of host defenses (6).

We describe a 2-month-old Hispanic girl with hemophagocytosis before the clinical diagnosis of GS was made. She did not have a mutation at the Griscelli locus. Her course was marked by temporary remissions on treatment. Because of a lack of a bone marrow donor, she was unable to undergo transplantation and died from her illness at age 5½ months. The literature concerning GS is reviewed.

## CASE REPORT

A 2-month-old Hispanic girl had a 3-day history of fever, hepatomegaly (6 cm), splenomegaly (5 cm) palpable below the costal margins, and a petechial rash. Her hemoglobin was 3.9 g/dL, platelet count was 14,000/μL, white blood cell count was 7,300/μL, absolute neutrophil count was 730/μL, aspartate aminotransferase was 201 U/L (n = 13–40 U/L), γ-glutamyltransferase was 339 U/L (n = 0–292 U/L), and lactate dehydrogenase was 1,189 U/L (n = 300–600 U/L). Her prothrombin time was 15.8 seconds (n = 11.5–14 seconds), activated partial thromboplastin time was 39 seconds (n = 21–36), fibrinogen degradation products were <5 μg/mL, and fibrinogen was 41 mg/dL (n = 127–409 mg/dL). Blood and urine bacterial and cytomegalovirus

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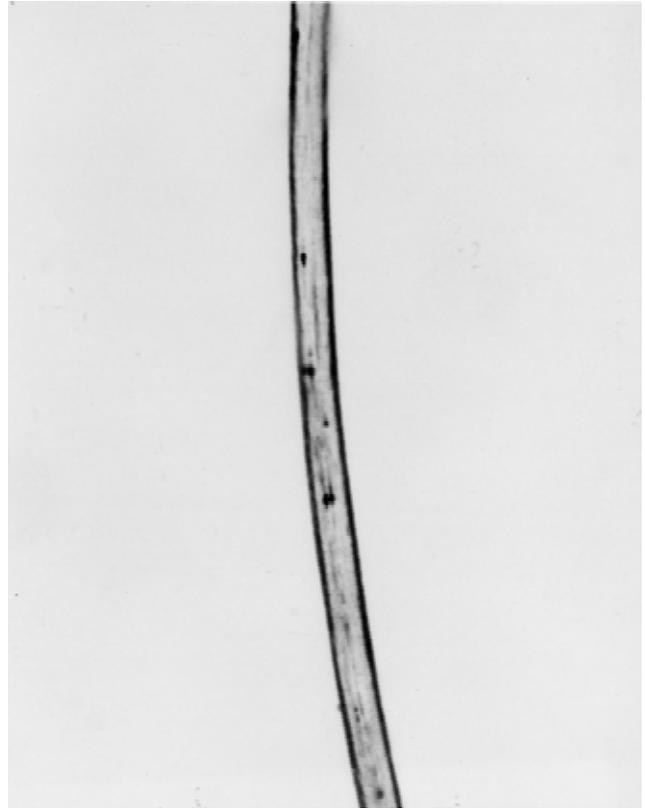
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cultures were negative. Antibody titers for Epstein–Barr virus, parvovirus, cytomegalovirus, hepatitis B and C, toxoplasma, syphilis, and human immune deficiency virus were not increased. Polymerase chain reaction studies of blood and bone marrow for Epstein–Barr virus, cytomegalovirus, and parvovirus were negative. Bone marrow aspirate showed a prominent histiocytic infiltrate with extensive hemophagocytosis confirmed on bone marrow biopsy staining with anti-CD68 antibody. Bone marrow aspirate flow cytometry and cytogenetics showed no evidence of clonal proliferation. She received blood, platelet, cryoprecipitate, and intravenous immunoglobulin, the latter at a dose of 1 g/kg on days 4 and 9 of hospitalization, but with no platelet increase. Partial albinism was acknowledged later because of silver-gray facial hair and unusually light scalp hair for her ethnic origin. There was no cutaneous or ocular albinism (Fig. 1). Chediak–Higashi, Hermansky–Pudlak, and Griscelli syndromes were therefore considered. Griscelli syndrome was confirmed by characteristic findings on microscopy of skin and hair biopsies. The hair showed unevenly clustered pigment in the medulla (Fig. 2). Fontana–Masson stain of the skin biopsy demonstrated accumulation of melanosomes (Fig. 3). DNA from cultured skin fibroblasts was analyzed for mutation at the Griscelli locus (4), but no known mutation was identified.

Peripheral blood flow cytometry showed 83% CD3-positive T lymphocytes, a normal CD4-to-CD8 ratio of 1.7, 10% NK cells, and 3% B-cells. Serum immunoglobulin level tests were not performed because of recent administration of intravenous immunoglobulin. Cell-mediated immunity assessed by mitogen stimulation of her lymphocytes was normal; in the mixed lymphocyte reaction the patient's cells did not stimulate allogeneic lymphocytes, despite the presence of human leukocyte DR antigens on her lymphocytes. Delayed hypersensitivity skin tests were not performed because of unreliability of these tests at a young age. Neutrophil function was normal by nitrobluetetrazolium reduction and chemiluminescence. Paternal family history



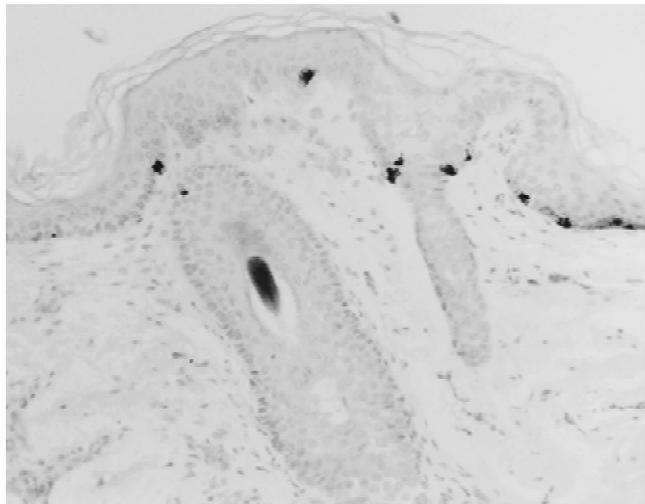
**FIG. 1.** Patient with GS depicting facial hair hypopigmentation.



**FIG. 2.** Hair showing unevenly clustered pigment in the medulla.

was unavailable; maternal family history was negative for a similar disease. The patient had no siblings.

On hospital day 10, because of severe persistent pancytopenia (white blood cell count 2,700/ $\mu$ L, absolute neutrophil count 54/ $\mu$ L, platelets 9,000/ $\mu$ L, and hemoglobin 10 g/dL maintained on red blood cell transfusions) she was administered intravenous etoposide 150 mg/m<sup>2</sup> per dose,



**FIG. 3.** Fontana–Masson stain of the skin biopsy demonstrating accumulation of melanosomes.

two times per week for 2 weeks. It was then administered weekly, plus dexamethasone 10 mg/m<sup>2</sup> per day orally for 2 weeks after tapering. Therapy was based on the Hemophagocytic Lymphohistiocytosis Study Group protocol (HLH-94). She started responding by day 6 of treatment and by day 20, she had an absolute neutrophil count of 903/ $\mu$ L, hemoglobin 10.6 g/dL, and platelets 421,000/ $\mu$ L. Her liver size shrunk to 2 cm below the costal margin, and the spleen became barely palpable. One month into her treatment, she experienced relapse with pancytopenia and liver and spleen both 7 cm below the costal margin. A repeat bone marrow aspirate showed severe granulocytic hypoplasia and persistent hemophagocytosis; no viral or bacterial infections were identified. Evaluation of her CNS showed normal cerebrospinal fluid cytology and chemistry; brain computed tomography showed mildly dilated ventricles and prominent sulci consistent with cortical atrophy.

After relapse, she was treated with an alternative regimen of etoposide at 5 mg/kg per day intravenous for 5 days, and daily oral cyclosporin (CSA) at 8 mg/kg per day. On day 5, she had involuntary movements of her head and neck while awake, without changes in sensorium, behavior, or general activity. Magnetic resonance imaging showed generalized atrophy and enlarged ventricles without focal lesions; her tremors were attributed to CSA compounded by hypomagnesemia. Serum magnesium level was 1.5 mg/dL (n = 1.7–2.4 mg/dL), and serum CSA levels were therapeutic at 280 ng/mL (n = 200–300 ng/mL). Because of lack of response after 3 weeks of etoposide and CSA, antithymocyte globulin at 10 mg/kg per day intravenous and methylprednisolone 5 mg/kg per day intravenous for 5 days were added. Her tremors continued to worsen and sequential electroencephalographs showed significant worsening, from focal slowing on each hemisphere to diffusely abnormal waveforms suggestive of cerebral dysfunction. In view of this deterioration and persistent pancytopenia (white blood cell count 1,000/ $\mu$ L, absolute neutrophil count 30/ $\mu$ L, platelets consistently <7,000/ $\mu$ L), CSA was stopped. The search for an unrelated human leukocyte antigen-matched bone marrow donor proved unsuccessful and a decision was made between the family and the health care providers to stop immunosuppressive treatments. The patient succumbed to her disease after a severe *Pseudomonas aeruginosa* septicemia at age 5½ months. The baby's mother consented to post-mortem needle biopsies of the bone marrow, liver, and spleen, all of which confirmed extensive infiltration with lymphocytes and macrophages showing hemophagocytosis.

## DISCUSSION

Griscelli syndrome was first described in 1978 and subsequently, there have been several reports concerning this syndrome (Table 1). It is an immunodeficiency with a highly variable primary immune defect. Decreases in T-cell and NK-cell cytotoxicity, reactions to tuberculin, streptokinase-streptodornase antigen, and *Candida* are found in most

cases (3). The lymphocytes proliferate in response to phytohemagglutinin, purified protein derivative, or *Candida*, and in a mixed lymphocyte reaction, normal proliferation occurs in response to allogenic lymphocytes; the stimulation capacity of the patient's lymphocytes is, however, decreased (3,12). The capacity of the NK cells to lyse target cells is impaired or absent. Consistent with previous reports, we found normal mitogen stimulation of lymphocytes, neutrophil function, and numbers of T cells, B cells, and NK cells in the peripheral blood. In the mixed lymphocyte reaction, the patients lymphocytes did not stimulate allogenic lymphocytes despite the presence of major histocompatibility complex class I and II molecules on their surface. We speculate that because of the dependence of endosomal and lysosomal sorting pathways of lymphocytes on *myosin-Va*, this defective function in GS may lead to improper placing of antigen and major histocompatibility complex class II molecules on the cell surface. Hence, responder T cells may not be able to efficiently recognize these "empty molecules." However, it is less clear that such speculation should apply to antigen-loading of major histocompatibility complex class I molecules. Natural killer cell function was not directly tested. Delayed type cutaneous hypersensitivity was not tested because of the patient's young age.

The Griscelli locus has been found to colocalize at *15q21* with an unconventional myosin gene locus (4). Mutations have been found at this site in two of three patients tested at different functional domains of the gene. It is thought that altered *myosin-5a* structure and function secondary to the mutation affects membrane transport and organelle trafficking. These are key processes in the function of different cells and are postulated to affect the T-cell-mediated immune responses. Our patient did not have a mutation in the *myosin-5a* gene. Using fine haplotype analysis, Pastural et al. (7) have recently identified involvement of a second gene in GS, distal to *MYO5a* (Griscelli syndrome 2). This gene localizes to approximately 1 to 7.3 cm of the *MYO5a* at the same locus (*15q21*). A pattern of clinical differences is emerging in the two groups with patients with mutated *myo-Va* and primary neurologic problems without accelerated phase of the disease. In patients without the *myo-Va* mutation, accelerated phases are common and neurologic problems are secondary to CNS involvement. Clinically, our patient fits into the latter group.

The clinical and microscopic features described in previous cases are fairly consistent. Most children are of Mediterranean or Middle Eastern origin (Turkish, African, Saudi Arabian, Italian, French) and born into consanguineous families; there is one Hispanic (8) and two whites reported (1,9). Our patient is of Hispanic origin, with no history of consanguinity. Patients generally receive diagnoses during infancy; the youngest patients described were 6 weeks old, 7 weeks old (8,9), and 3 months old (14,17,18). Our patient would be among the youngest at 2 months old. All patients have a silver-gray sheen to their hair. This is the single most consistent cutaneous expression of albinism in these pa-

TABLE 1. Reports on Griscelli syndrome

Ref	Year	No of pts	Race/con	Age/sex	Clinical features				Immune defect	Specific treatment	Outcome
					Silver hair	HSM	Pancytopenia	CNS disease			
1	1978	2	Af/+ C/-	11y/F 5mo/F	+ +	+ +	- -	- -	↓ IgG, IgA, -ab to DPT DTH-, abn MLR	Levamisol Vitamin C Steroids	A at 11y Dead
2	1978	1	It/+	4y/M	+	+	-	-	Defective bactericidal function of neutrophils	None	Dead
18	1980	1	Nm/+	3mo/M	+	+	+	-	-DTH, abn MLR	Steroids	Nm
17	1990	1	Arab/+	3mo/F	+	-	-	-	None	BMT	A at 22 mo
13	1991	1	Turk/+	9mo/M	+	+	+	+	↓ IgG2, abn MLR, -DTH	VP16, steroids	Dead
19	1992	12	Arab/+	40d-5y 6M, 6F	+	+9/12	+9/12	+7/12	-DTH, abn Neutrophil function 7/12, low response of T cells to mitogens 8/12, abn MRL 4/12	MTX, steroids, Vitamin C	5/12 A
11	1993	4	Arab/+	4y/M, 9mo/F, 3y/F, 7mo/F	+, +, +	+, Nm, Nm	- Nm, Nm	+, +, +	None Nm Nm Nm	Vitamin C Acyclovir	1/4 A at 4 y
15	1994	1	Turk/Nm	9y/F	+	+	+	+	Nm	VP16, steroids	Dead
3	1994	7	It/+, Fr/- Turk/+, Af/+, 4y/F, 3.5y/M, 4.5y/F, 3.5/M	4mo/M, 2y/F, 3y/F, 4y/F, 3.5y/M, 4.5y/F, 3.5/M	+, +, +, +, +, +, +	-, -, -, -, -, -, -	+, +, +, +, +, +, +	+, +, +, +, +, +, +	-DTH, abn MLR, ↓ NK function, abn neutrophil function.	BMT in 3, Steroids, VP16, CSA, VCR, ATG,	3/7 A
14	1995	3	Turk/+	5y/F, 9mo/M 3mo/M	+, +, +	-, +, +	+, -, -	+, +, -	-DTH	VP16, steroids	1/3 A
9	1997	1	German/+	7wk/M	+	+	+	+	None	IVIG, steroids	Dead
12	1998	1	Turk/+	5mo/M	+	+	+	+	None	Steroids, IVIG	Dead
8	1998	1	Hisp/+	6wk/F	+	+	+	+	↓ CD19, ↓ NK function	Steroids, IT MTX	Dead
10	1999	1	Turk/+	4y/F	+	+	+	+	Nm	Steroids, VP16, BMT	A at 18 mo
Self	2000	1	Hisp/-	2mo/F	+	+	+	+	Abn MLR	IVIG, VP16, steroids, CSA, ATG	Dead

Ref = reference, Con = consanguinity, Af = African, C = Caucasian, It = Italian, Fr = French, Hisp = Hispanic, + = present, - = absent, Nm = not mentioned, ↓ = decreased, wk = weeks, HSM = hepatosplenomegaly, CNS = central nervous system, IgG = immunoglobulin G, IgA = immunoglobulin A, ab = antibody, DPT = diphtheria pertusis tetanus, DTH = delayed type cutaneous hypersensitivity, abn = abnormal, MLR = mixed lymphocyte reaction, NK = natural killer cells, CSA = cyclosporin, VCR = vincristine, ATG = antithymocyte globulin, IVIG = intravenous immunoglobulin, IT MTX = intrathecal methotrexate, BMT = bone marrow transplantation, A = alive.

tients. It can be subtle and easily overlooked. Commonly, skin and eye hypopigmentation are not evident. Our patient received diagnosis of GS after 7 to 10 days of hospitalization when the odd color of her facial and scalp hair was noted.

Light microscopy of the hair shaft in GS shows uneven clusters of pigment with predominantly large, dark granules in the medulla. Fontana-Masson-stained sections of the skin showed accumulation of melanosomes in melanocytes contrasting to poorly pigmented adjacent keratinocytes. It is thought that the transfer of pigment from melanocytes to keratinocytes is defective secondary to a more global defect

of intracellular trafficking. The melanin itself was normal and electron microscopy showed numerous stage IV melanosomes in the epidermal melanocytes. The melanocytes appeared to have normal-appearing dendritic processes expanding into intercellular spaces. Normal-appearing Langerhans cells are present in the skin (3). Our patient had findings consistent with GS on microscopic examination of her hair and skin (Figs. 2,3).

Secondary to their immune defect, these patients experience a virus-induced "accelerated phase," commonly caused by Epstein-Barr virus. This can start at any age (mean age at onset 36 months) and consists of features of

hemophagocytosis, i.e., hepatosplenomegaly, pancytopenia, disseminated intravascular coagulation, and hypertriglyceridemia. The presenting feature in our patient was fulminant hemophagocytosis; GS was diagnosed subsequently. Even though no infectious agent was identified in our patient, the postmortem findings were typical for hemophagocytosis.

Another devastating complication reported in most patients at a later stage is the onset of neurologic problems caused by lymphohistiocytosis of the CNS (19). Manifestations include convulsions, increased intracranial pressure, cerebellar signs, spasticity and hemiparesis. Cerebrospinal fluid may show increased protein and cell count or may be normal. Electroencephalographs and radiologic findings are nonspecific. Recently, the human herpes virus 6 has been implicated as a causative agent for CNS disease (8). Our patient had significant tremors that were initially attributed to cyclosporin and hypomagnesemia. Her cerebrospinal fluid studies were normal, including cultures for viruses, bacteria, and fungi. The magnetic resonance imaging findings of mild ventricular dilatation secondary to cerebral atrophy and the diffuse changes in electroencephalographs both indicate a generalized CNS process probably secondary to lymphohistiocytic infiltration; unfortunately, brain autopsy is unavailable for confirmation. There is a recent report (10) of a patient who underwent an allogeneic-matched sibling bone marrow transplantation with complete resolution of neurologic sequelae; most reported patients have died when severe neurologic sequelae had set in (7,11). An uncommon presentation of the disease recently described is a myelodysplastic syndrome of refractory anemia with excessive blasts (12).

The mean age at death is 5 years, and death is secondary to severe neurologic sequelae or recurrent infections. Supportive care includes antibiotics and transfusions. Specific therapy for hemophagocytosis includes etoposide, steroids, and intrathecal methotrexate (3,13–15). Intravenous immunoglobulin and methylprednisolone were used in one reported patient, but the outcome was fatal (9). We initially used intravenous immunoglobulin, etoposide, and steroids; however, because of severe refractory thrombocytopenia, we did not administer intrathecal medications. Subsequently, because of refractoriness to treatment, she was administered CSA, antithymocyte globulin, and methylprednisolone, as previously described (16), without any response. Bone marrow transplantation appears to be the only curative therapy and is the treatment of choice when a human leukocyte antigen-matched sibling donor is available (3,10,17).

In summary, we describe a case of GS with some atypical features. The age of onset was early (2 months old). Hispanic ethnicity and absence of consanguinity were unusual. There was no detectable mutation at the Griscelli locus. Her subtle pigmentary abnormalities led to some difficulty in the diagnosis of GS initially, thus highlighting the importance of considering GS in all cases of infantile hemophagocytosis.

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