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Abstract

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Premature birth, respiratory distress, intracerebral hemorrhage and silvery-gray hair: differential diagnosis of the three types of Griscelli syndrome

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Running title: Griscelli syndrome: differential diagnosis

Abbreviations: GS=Griscelli syndrome, MYO5A=gene of myosin 5 a

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Abstract

A preterm neonate, born to consanguineous parents, presented with respiratory distress, intracerebral hemorrhage and a silvery-gray sheen of the hair and eyelashes. Griscelli syndrome (GS) type 3 was diagnosed following the detection of a novel homozygous mutation of the melanophilin gene. Thus, only the hypopigmentation, but not the patient’s other clinical features, were attributable to this form of GS. Differential diagnosis of the various forms of GS must be performed as early as possible since GS2 is associated with a life-threatening but curable immune disorder.
**Introduction**

Griscelli syndrome (GS) is a rare autosomal recessive disorder characterized by hypopigmentation of the skin and hair, the presence of large clumps of pigment in hair shafts and an accumulation of mature melanosomes within the melanocytes (1). These pigmentary features are specific for patients with GS and can be distinguished from the hypopigmentation observed in other disorders such as Chediak-Higashi and Hermansky-Pudlak syndromes. Griscelli syndrome was first described as the combination of an immune disorder to this hypopigmentation. Most GS patients develop an uncontrolled T lymphocyte and macrophage activation syndrome (known as the “accelerated phase” or hemophagocytic syndrome) which may lead to secondary neurological impairment. In contrast, other patients develop a primary severe neurological impairment in the absence of apparent immune abnormalities. Defects in two different genes, *MYO5A* and *RAB27A*, are thought to be responsible for GS type 1 and 2 (GS1 and GS2) phenotypes, respectively (2, 3).

In GS1 the characteristic hypopigmentation is combined with a severe primary neurological impairment which arises early in life. These patients carry mutations in the myosin 5A gene (*MYO5A*), which encodes an organelle motor protein (myosin Va) with a critical role in neuron function. In contrast, hypopigmentation in GS2 is associated with an immune disorder which leads to episodes of hemophagocytic syndrome with activated T cells and macrophages infiltrating various organs (including the brain) and causing massive tissue damage, organ failure, pancytopenia and (in the absence of
immunosuppressive treatment) death. Bone marrow transplantation is the only curative treatment for this condition. Griscelli syndrome type 2, caused by mutations in the RAB27A gene encoding a small GTPase protein (Rab27a) involved a vesicular secretory pathway. The immune deregulation observed in GS2 patients results from the absolute requirement for functional Rab27a in lymphocyte cytotoxic granule release and the critical role of this cytotoxic pathway in lymphocyte homeostasis. The MYO5A and RAB27A genes both map to the same chromosomal region (15q21.1). More recently, a third form of GS (type 3, GS3) was shown to result from a melanophilin defect. In this genetic form, the phenotype is restricted to the hypopigmentation that is characteristic of GS. Melanophilin is an effector of Rab27a in melanocytes. The Rab27a protein targets the melanosome membrane and binds to melanophilin. Melanophilin then recruits the molecular motor myosin Va, which allows movement or tethering of the melanosomes on the actin cytoskeleton. The tripartite myosin Va-melanophilin-Rab27a complex drives mature melanosomes to the dendritic tips of melanocytes and thus enables delivery of the melanin to adjacent keratinocytes. These findings explain the common pigmentary features observed in GS1, GS2, and GS3 patients whose distinct characteristics (listed in Fig. 1A) are partly due to tissue-specific expression of the corresponding genes. Although all three proteins are expressed in melanocytes, only myosin Va is expressed in neurons and only Rab27a is expressed in cytotoxic lymphocytes.

Here, we report on a preterm neonate with GS-typical hypopigmentation and who presented with respiratory distress and intracerebral hemorrhage. A mutation in the melanophilin gene was found and so GS3 was diagnosed. Since GS3 is restricted to
hypopigmentation, no treatment was needed. The preterm delivery, respiratory distress and intracerebral hemorrhage were not attributable to hemophagocytic syndrome and were unrelated to GS3.
**Patient presentation**

We report on a Saudi-Arabian boy, now aged 19 months. He was born to consanguineous healthy parents (first degree cousins). There was no history of childhood death or suspected immunodeficiency in the family. Due to premature rupture of the fetal membranes, the neonate was delivered after 30 weeks of gestation. The weight was 1010 g (10\textsuperscript{th} percentile for gestational age), length 38 cm (10\textsuperscript{th} percentile) and head circumference 26 cm (just below the 10\textsuperscript{th} percentile). Due to infant respiratory distress syndrome, the baby was taken to a neonatal intensive care unit and received mechanical ventilation for 58 days. A grade II intraventricular, cerebral hemorrhage and a grade IV hematoma (both on the left hand side) were diagnosed by ultrasonography at the chronological age of 9 days. Hemostatic plasma parameters and blood cell counts were normal. The patient did not have fever, lymphadenopathy or hepatosplenomegaly.

Regularly performed neurological assessments showed normal muscle tone, gross motor activities and cognitive functions. A computed tomography (CT) scan of the brain at the age of 7 months showed no shift of the brain midline structures and no signs of recent intracranial hemorrhage. There was evidence of mild lateral ventricle asymmetry that could have resulted from the previous hemorrhage. At the chronological age of 10 months, the child had a developmental age of 8 months.

The boy had silvery-gray hair and fair skin as shown in Figure 1 B. He was admitted to the Immunology department for suspected GS. Light microscopy examination of his hair showed large clumps of pigment irregularly distributed along the
hair shaft (Fig. 1 C). There were no episodes suggestive of hemophagocytic syndrome. Segregation of microsatellite markers in the family excluded the RAB27A/MYO5A locus but was compatible with the MLHP locus. Genomic DNA sequencing revealed a novel, homozygous mutation in MLHP exon 7 (delC986) leading to a frame shift and L344X (Figure 2 A). Both parents were heterozygous. The L344 residue is located in the myosin-binding domain of melanophilin (Figure 2 B).

Informed consent for the present study and publication of the photographs were obtained from the child's parents and the work was approved by the local independent ethics committee.
Discussion

Here, we report on a boy with a new homozygous mutation in the melanophilin gene which resulted in GS3, where the GS phenotype is limited to hypopigmentation. This is the second case of GS3 reported. Our preterm born patient had GS-characteristic hypopigmentation, was suffering from infant respiratory distress syndrome and had radiological signs of intraventricular hemorrhage. He recovered from the respiratory distress and did not show any neurological sequelae at the age of 8 months. Characteristic hypopigmentation is a shared feature in individuals with GS1, GS2, and GS3. In GS1, hypopigmentation is combined with a severe neurological impairment and muscle hypotonia at onset. Primary mental retardation and regressive neurological disorders have been described (4). The child described here did not show any of these clinical signs and his mental development was normal.

Griscelli syndrome type 2 is associated with an immune disease which results in episodes of a life-threatening hemophagocytic syndrome that necessitate treatment with immunosuppressive agents. In GS2, the age at the first episode of hemophagocytic syndrome varies. Hemophagocytic syndromes can occur in the neonatal period and may be associated with preterm delivery (5). Thus, GS2-associated hemophagocytic syndrome represents an important differential diagnosis in a the patient with GS-characteristic hypopigmentation, preterm delivery, respiratory distress and intraventricular hemorrhage, such as the case reported here. It is clear that prematurity per se is not related to GS1 or GS3; however, it can be associated with hemophagocytosis and thus GS2.
In GS2, the only curative treatment for the immune disease is hematopoietic stem cell transplantation. Early diagnosis and transplantation are essential for a positive outcome in GS2 patients and it has been suggested that pre-emptive transplantation, i.e. before the occurrence of the hemophagocytic syndrome should be preferred. We diagnosed GS3 in the patient presented here. Preterm delivery, distress and intraventricular hemorrhage were not associated with a hemophagocytic syndrome. Hence, neither immunosuppressive treatment nor hematopoietic transplantation was indicated in this patient. Hemophagocytosis is characterized by unremitting polyclonal CD8 T cell activation and is associated with genetic defects in cytotoxicity. The pathogenesis of hemophagocytosis is based on the cytotoxic effector cells’ inability to kill the antigen presenting cells and thus eliminate the infecting pathogens. Persistence of the trigger probably induces an unremitting polyclonal CD8 T cell activation. It has been shown, that the gene product of melanophilin is not detectable in cytotoxic T lymphocytes (in contrast to melanocytes). Therefore, a melanophilin deficiency will not affect the T lymphocytes’ cytotoxic machinery. This is why the child presented in this report does not have a greater risk of developing hemophagocytosis than a healthy child lacking the underlying genetic defect. This view is further supported by the fact that the proband with melanophilin deficiency reported previously is now 21 and has not experienced any hemophagocytosis. Additionally, no hemophagocytosis has occurred in our own, unpublished cohort of 3 further individuals with melanophilin deficiency (current ages - patient 1: 19 years, patient 2: 7.6 years, patient 3: 4 years).
We hope that the present case report will draw the attention to GS, since although GS2 is associated with a life-threatening and potentially curable immune disorder, GS1 and GS3 do not require hematopoietic stem cell transplantation. General pediatricians are not very familiar with GS. Although the syndrome is rare, silvery hair sheen is easy to recognize. Hence, recognition of this feature by the general pediatrician and referral to a pediatric immunologist could improve life expectancy and quality of life for patients suffering from GS2.
References


**Figure legends**

Figure 1: Summary of the three different forms of GS (A); HLH = hemophagocytic lymphohistiocytosis; * = not present in a patient with Myo Va F-exon deletion. Patient with GS3 showing (A) the characteristic silvery gray sheen of the hair and eyelash, and (B) the characteristic large clump of pigment in the hair shaft observed by microscopic analysis.

Figure 2: (A) *MLPH* mutation in the patient. Detection of *MLPH* mutation was done by fluorometric sequencing. DNA sequence analysis of exon 7 showed a delC986 leading to L344X (homozygous for the patient). Mother and father are heterozygous for the base pair deletion. (B) Localization of the nonsense mutation on the schematic representation of melanophilin: Melanophilin is composed of three domain; a SHD domain (black box), a myosin binding domain (MBD)(hatched box) and an actin binding domain (ABD)(hatched box). The patient mutation L344X is located in the MBD domain.
### The three forms of Griscelli syndrome

<table>
<thead>
<tr>
<th>Griscelli syndrome</th>
<th>Silvery hair sheen</th>
<th>Clinical presentation</th>
<th>Neurological involvement</th>
<th>HLH</th>
<th>Genetic defect</th>
<th>Ref.</th>
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<td>+</td>
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<td>+</td>
<td>-</td>
<td>Myosin Va</td>
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<tr>
<td>Type 2</td>
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<td>+ (due to HLH)</td>
<td>-</td>
<td>+</td>
<td>Rab27a</td>
<td>4, 13, 16</td>
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<tr>
<td>Type 3</td>
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<td>-</td>
<td>-</td>
<td>-</td>
<td>Melanophilin</td>
<td>10</td>
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</table>

**Figure 1**

A. Table showing the three forms of Griscelli syndrome with details on clinical presentation, neurological involvement, HLH, and genetic defect.

B. An image of a baby with 'Griscelli syndrome hair' and normal brown and blond hair for comparison.

C. Images showing normal brown hair, normal blond hair, and 'Griscelli syndrome hair'.
Figure 2

A

WT sequence:

CATTC

CATTC

CATTC

981

CATTC

CATTC

CATTC

Del C986

Mother

Father

Patient

B

Melanophilin:

SHD

MBD

ABD

L344X

Rab27a

Myosin

Actin

- S1p homology domain
- Myosin binding domain
- Actin binding domain