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Phenotype with MYO-5A exon-F deletion**

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Griscelli Syndrome Type 3-like Phenotype with MYO-5A exon-F deletion

Abstract

Griscelli Syndrome (GS), a rare autosomal recessive disorder characterized by partial albinism and additional features of immunological impairment or severe neurological impairment, results from mutations in the *MYO5A* (GS1), *RAB27A* (GS2), or *MLPH* (GS3) genes. Manifestations of GS3 are restricted to hypopigmentation of the skin and silvery grey hair. The identical phenotype has been described in a patient with *MYO5A* F-exon deletion. Here we report the second case with clinical manifestations of GS3 resulting from the same genomic deletion in *MYO5A* gene. The presented patient provides additional evidence for and supports the fact that *MYO5A* F-exon deletion results in GS3 phenotype.

Key Words Griscelli Syndrome, *MYO5A*, F-exon

Introduction

Griscelli syndrome (GS) is a rare autosomal recessive genetic disorder characterized by skin or hair hypopigmentation, with the presence of large clumps of pigment in hair shafts and an abnormal accumulation of melanosomes in the center of melanocytes (1). Neurologic abnormalities, variable cellular immunodeficiency and an accelerated phase of uncontrolled T lymphocyte and macrophage activation may be seen as additional features (2,3).

Different phenotypes observed in each GS form are related to the function and tissue expression of defective proteins. Three forms according to the underlying molecular defect have been defined so far. Griscelli syndrome type 1 (GS1) manifests with primary dysfunction of central nervous system. These patients carry mutations in myosin 5A gene (*MYO5A*) (2). Type 2 (GS2) is associated with an immune defect, leading to episodes of uncontrolled T lymphocyte and macrophage activation syndrome known as accelerated phase or hemophagocytic syndrome (HS). It is characterized by hepatosplenomegaly, fever, pancytopenia, haemorrhages, elevated levels of transaminases and triglycerides (4). Hematopoietic stem cell transplantation is the only treatment for this fatal condition. This form results from mutation in the *RAB27A* gene. Type 3 (GS3) has a phenotype restricted to hypopigmentation without any immunologic or neurologic manifestations and is caused by mutation in the melanophilin (*MLPH*) gene (5). An identical GS3-like phenotype has been described in a patient with deletion of the exon F of *MYO5A* (5). Several isoforms of *MYO5A* have been characterized in both mice (6) and humans (5). They result from different combinations of spliced exons, along with the F-exon, which

encode part of the tail region of MYO5A. Absence of MYO5A accounts for the primary severe neurological impairment observed in GS1. Normal neurological development and functions require the presence of MYO5A, at least in its short F-exon–spliced isoform. The fact that brain transcripts lack the F-exon may explain the absence of neurological symptoms in patients with MYO5A F-exon deletion (7,8).

To date, only one patient with hypopigmentation of the skin and silvery-gray hair caused by MYO5A F-exon deletion has been reported (5). Here we report a second case with clinical manifestations of GS3 resulted from the same deletion in the *MYO5A* gene.

Case report

A 7-month old boy was first admitted with the complaints of eczema and wheezing. The parents are first degree cousins. First pregnancy resulted in abortion. The first child having dark hair died at the age of 4 years old with the complaints of atopic dermatitis and chronic diarrhea started at age 6 months possibly due to food allergy. Present case, the product of third pregnancy, was admitted to the hospital at the age of 5 months with symptoms of coughing and wheezing. He was given inhaler bronchodilator and steroid therapy in addition to antibacterial therapy. At 6 months of age he had severe allergic reaction with cow's milk. He was again hospitalized at age 7 months with the diagnosis of pneumonia and bronchial hyperreactivity. Laboratory results showed leukocytosis and positive anti CMV IgM antibody. Physical examination revealed silvery grey hair and eyebrow and eyelashes, and eczema. His development was normal. The neurological examination was unremarkable. Microscopic examination of his hair shafts showed large discrete clumps of melanin pigment along with the length of the shaft consistent with the diagnosis of Griscelli syndrome (Figure 1). On laboratory examination, the haemoglobin level was 10.8 gm/dl, the white blood cell count was 20.4×10^9 /L, platelet count was 240×10^9 L. Immunologic workup data are given in Table 1. The blood biochemistry results were normal. Cow's milk specific IgE and specific IgE for other common food allergens (peanuts, egg, wheat, banana) were positive. Skin prick testing confirmed that he was sensitized to a number of foods, including cow's milk, peanut and egg white.

He was referred with the diagnosis of GS to Hacettepe University Pediatric Immunology clinic at the age of 8 months. When seen he had eczema and diaper dermatitis in addition to partial albinism. His psychomotor development was compatible with his age and organomegaly was not recorded. He had leukocytosis and eosinophilia (Table 1). Stool pH was 6 and reductant substance and steactocrits were negative. Bone marrow aspiration smear showed hypercellularity, rare erythroid vacuolization and eosinophilia (18%), hemophagocytosis was not observed. He was given hydrolyzed formula. During follow-up he had mild eczema, mild eosinophilia and high serum IgE levels (Table 1). He is now 3 years old, and did not have any problems except food allergy.

Genetic analysis

Using segregation linkage analysis, the *GS3* locus was excluded in this family. In contrast, the segregation analysis of the polymorphic markers linked to the 15q21 *GS1* and *GS2* locus identified a homozygous haplotype in the patient, indicating that *Rab27a* and *MYO5A* are potential candidate genes. Mutation screening of the two causative genes was undertaken in the patient. No mutation could be identified in the *RAB27A* gene. Sequencing of *MYO5A* identified a homozygous 2,439bp genomic deletion (g.189721_192160del) spanning the whole F-exon sequence, which is identical to the deletion previously reported in another patient (5).

Discussion

Griscelli Syndrome results from mutations in *MYO5A*, *RAB27A*, and *MLPH* which encode proteins that form a complex shown to play role in the capture and local movement of melanosomes in the actin-rich cell periphery of melanocytes (4, 5). The mouse models of *GS1*, *GS2*, and *GS3* (dilute, ashén, and leaden mice, respectively) have been extensively studied for the underlying pathology of *GS*, and helped approach the role of *MYO5A* in neurons, the mechanisms of melanosome transfer to keratinocytes, and the role of *Rab27a* in the immune system (9).

Component *RAB27A* interacts with the melanosomal membrane, where it serves as a receptor for specific effectors. In melanocytes, membrane-bound *RAB27A* recruits its downstream effector *MLPH*. Subsequently, *MLPH* is able to bind actin, as well as the C terminal globular tail domain of the actin-associated motor protein *MYO5A*, resulting in the capture of mature melanosomes to the peripheral actin network. If any member of the *RAB27A*-*MLPH*-*MYO5A* tripartite complex is defective, melanosome transport is impaired. As a result, melanosomes are not captured in the cell periphery, not transferred from melanocytes to adjacent keratinocytes, and accumulate in the perinuclear region of melanocytes, resulting in the hypopigmentation and silvery-gray appearance of the patients' hair (10).

The heterogeneity in the features observed in each *GS* form is in accordance with the functions and tissue expression of the respective proteins (3). Apart from hypopigmentation of the hair and skin, *GS1* resulting from *MYO5A* deficiency is associated with severe neurological impairment with no immunodeficiency (2). One patient with *GS* hypopigmentation characteristics but without neurological symptoms carrying deletion of exon-F of the *MYO5A* has been previously reported (5). We describe here a second case of *GS1* with a phenotype restricted to hypopigmentation as a result of the same *MYO5A* exon-F deletion. Our data provides additional evidence for and supports the fact that exon F of *MYO5A* is required for the function of the *Rab27a*-*MLPH*-*MYO5A* tripartite complex in melanocyte, but dispensable for *MYO5A* function in neurologic development and behaviour.

Griscelli Syndrome type 2, the most common form of *GS*, is caused by mutations in *RAB27A* located on chromosome 15q21 (2). *Rab27a* has very strong expression in melanocytes and

lymphocytes, but no expression in the brain. As RAB27 has also an important role in the exocytosis of cytotoxic granules in T and NK lymphocytes, in addition to hypopigmentation, GS2 patients have also an immune defect (3,4,9).

Griscelli Syndrome type 3 is caused by mutations in MLPH located on chromosome 2q37.3 leading to defective RAB27A-MLPH interaction (5). GS3 manifestations are restricted to hypopigmentation of the skin and silvery-gray hair. There is no neurological symptom and immune defect in this type of GS (5, 7, 10). These patients may remain undiagnosed since the only symptom is the pigmentary dilution.

In conclusion, we presented a patient who has MYO5A F-exon deletion with a phenotype restricted to hypopigmentation, similar to GS3 phenotype. Molecular analysis has utmost importance in patients diagnosed with Griscelli syndrome since treatment options, prognosis, and genetic counselling markedly differ among different types of the disease.

Conflict of Interest

None of the authors has any potential financial conflict of interest related to this manuscript.

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Table 1 Immunologic findings of the patient.

Patient/ Age	7 months	8 months	18 months	21 months
Hb (g/dl)	10.8	11.4	11.4	11.7
WBC (/mm ³)	20400	46500	10400	15900
Plt (/mm ³)	240000	640000	270000	421000
ALC (/mm ³)	7600 (3800-9900)	18600 (3800-9900)	5200 (1700-5900)	6500 (1700-5900)
ANC (/mm ³)	8000	9300	3000	7200
AEC (/mm ³)	510	13950	700	800
Immunoglobulins				
Ig A (g/dl)	201 (7-123)	68 (7-123)	-	202 (30-107)
Ig G (g/dl)	846 (304-1231)	2430 (304-1231)	-	1080 (605-1430)
Ig M (g/dl)	25 (32-203)	353 (32-203)	-	51 (66-228)
Ig E (IU/L)	657	726	-	1314
Lymphocyte subsets				
CD3 (%) (/mm ³)	51 (50-77)	56 (50-77)	-	-
	3876 (2400-6900)	10416 (2400-6900)		
CD4 (%) (/mm ³)	39 (33-58)	21 (33-58)	-	-
	2964 (1400-5100)	3906 (1400-5100)		
CD8 (%) (/mm ³)	15 (13-26)	22 (13-26)	-	-
	1140 (600-2200)	4092 (600-2200)		
CD19(%) (/mm ³)	34 (13-35)	33(13-35)	-	-
	2584 (700-2500)	6138 (700-2500)		
CD16+56 (%) (/mm ³)	13 (2-13)	13 (2-13)	-	-
	988 (100-1000)	2418 (100-1000)		
Degranulation test	Normal			

ALC, absolute lymphocyte count; ANC, absolute neutrophil count; AEC, absolute eosinophil count.

Figure 1 Patient's hair showed clumps of pigment in the hair shaft characteristic of Griscelli Syndrome.

