

ORIGINAL RESEARCH

POLARIZED LIGHT MICROSCOPY OF HAIR SHAFTS AIDS IN THE DIFFERENTIAL DIAGNOSIS OF CHÉDIAC-HIGASHI AND GRISCELLI-PRUNIERAS SYNDROMES

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Valente NYS, Machado MCMR, Boggio P, Alves ACF, Bergonse FN, Casella E, Moraes Vasconcelos D, Grumach AS and Oliveira ZNP. Polarized light microscopy of hair shafts aids in the differential diagnosis of Chédiak-Higashi and Griscelli-Prunieras syndromes. Clinics. 2006;61(4):327-32.

PURPOSE: To study and compare the appearance of hairs from patients with Chédiak-Higashi and Griscelli-Prunieras syndromes under light and polarized light microscopy.

METHOD: Hairs from 2 Chédiak-Higashi and 2 Griscelli-Prunieras patients were obtained and examined under normal and polarized light microscopy.

RESULTS: Under light microscopy, hairs from Chédiak-Higashi patients presented evenly distributed, regular melanin granules, larger than those seen in normal hairs. Under polarized light microscopy, shafts exhibited a bright and polychromatic refringence appearance. In contrast, hair from Griscelli-Prunieras patients, under light microscopy, exhibited bigger and irregular melanin granules, distributed mainly near the medulla. Under polarized light microscopy, shafts appeared monotonously white.

CONCLUSION: Light microscopic examination of hair shafts of patients with Chédiak-Higashi or Griscelli-Prunieras syndrome reveals subtle differences that are useful in identifying both disorders, but not in distinguishing between them. We provide evidence that polarized light microscopy of hair shafts, an approach that has not been previously described, aids in differentiating between these syndromes. We propose hair study by polarized light microscopy as a helpful complementary diagnostic method for differential diagnosis between CHS and GPS, especially when the more sophisticated molecular studies are not available.

KEY WORDS: Chédiak-Higashi; Griscelli-Prunieras; Silvery hair; Polarized light microscopy.

INTRODUCTION

Chédiak-Higashi syndrome (CHS) is a very rare genetic disorder, inherited in an autosomal recessive mode. It is clinically characterized by mild pigmentary dilution, silvery-blond hair, severe phagocytic immune deficiency, bleeding tendencies, recurrent pyogenic infection, progres-

sive neurological defects, and early death from a lymphoma-like hemophagocytic syndrome.¹⁻⁵

In 1978, Griscelli and Prunieras reported an autosomal recessive syndrome having clinical features and a course that resembled CHS, leading to the term, Chédiak-Higashi-like syndrome.^{6,7} Nowadays, it is a well characterized entity known as Griscelli-Prunieras syndrome (GPS).

The correct diagnosis and differentiation between CHS and GPS requires light microscopic examination (and, when possible, electron microscopic study) of skin and hair shafts, as well as immunological and peripheral blood smear evaluations. Determinations for specific genetic defects for each syndrome are currently available.

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The aim of this paper is to describe the polarized microscopic aspect of hair shafts from patients with CHS and with GPS. To the best of our knowledge, there are no previous reports in the English literature focusing on hair appearance under polarized light microscopy in these diseases.

METHODS

Case reports

Case 1:

A 20-month-old black girl, born to consanguineous (first-degree cousins) parents, presented since birth with generalized skin hypopigmentation and ash-gray hair. During follow-up, sun-exposed areas of skin have become darker, although flexures and the trunk have remained hypochromic. At the age of 8 months, she developed recurrent bacterial pneumonias. Under light microscopic skin examination, many large melanin granules were detected throughout the epidermis. Blood smear evaluation showed the presence of giant peroxidase-positive granules within granulocytes (Fig. 1), confirming the CHS diagnosis.

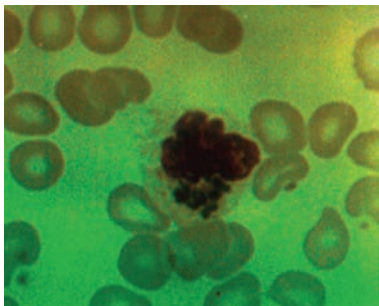


Figure 1 - CHS: blood smear with giant cytoplasmic granules inside the neutrophils (case 1).

Case 2:

A 5-year-old white female, born to nonconsanguineous parents, presented with generalized skin hypopigmentation and silvery hair since birth. By the age of 4 months, her skin became darker on the face and light-brown with a grayish hue on the extremities, sparing flexural areas. Otherwise, she was in good health. Light microscopic skin study of a hyperpigmented area on the forearm showed many large melanin granules inside melanocytes and keratinocytes. Peripheral blood smear examination, in which large peroxidase-positive granules inside neutrophils and lymphocytes were found, led to the CHS diagnosis.

Immunological data showed hypergammaglobulinemia and reduced stimulated chemotaxis in both CHS patients (Table I).

Case 3:

A 7-year-old white boy, born to first-cousin parents, was referred to our clinic due to silvery hair and a history of recurrent respiratory infections. The physical and neurological examinations were normal. Light microscopic skin examination (on skin of normal appearance) revealed dense hyperpigmentation of basal melanocytes, little pigment in the surrounding epidermis, and absence of giant melanin granules. The peripheral blood smear investigation was normal. Immunological data showed an impaired stimulated NBT (nitroblue tetrazolium) test (Table I). Clinical and laboratory data suggested a GPS diagnosis.

Case 4:

A 4-year-old white male, born to a nonconsanguineous couple, was admitted due to a progressive sleepy state. On physical examination, silvery-gray hair was noted (Fig. 2), and we were asked to evaluate him. A skin biopsy showed dense hyperpigmented basal melanocytes with sparse pigmentation in adjacent keratinocytes. Immunological studies showed low plasma levels of IgA and IgG for the patient's age (Table I). The peripheral blood smear was normal. He evolved with severe motor neurological deterioration, recurrent infections, and a progressive hepatosplenomegaly associated with myeloid metaplasia, leading to introduction of immunosuppressive therapy. With these clinical, skin, and hair microscopic findings, a GPS diagnosis was proposed.

At least 20 hair shafts from each of these 4 patients (2 females with CHS and 2 males with GPS) were collected and prepared on a glass slide with Entellan resin and a glass coverslip. Light microscopy examination, with and without polarization, was then performed.



Figure 2 - GPS: silvery hair (case 4)

RESULTS

Light microscopic hair examination

CHS patients: shafts showed evenly distributed melanin granules of regular diameter that were bigger than those seen in normal hair (Fig. 3).

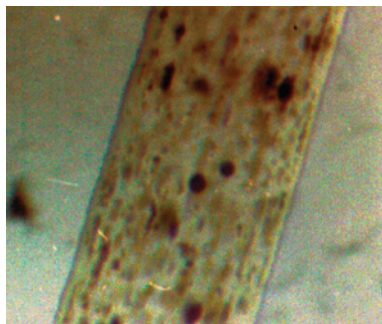


Figure 3 - CHS: hair under light microscopy (x100).

GPS patients: shafts presented large unevenly melanin granules, mostly located in vicinity of the medullar zone. They were larger than those described for normal hair as well as those seen in CHS hair (Fig. 4).

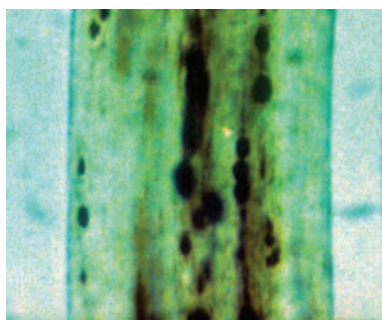


Figure 4 - GPS: hair light microscopy (x100).

Polarized light microscopic hair examination

CHS patients: bright shafts with different individual colors were detected, resembling normal white hairs (Fig. 5).

GPS patients: shafts looked bright with a monotonously whitish appearance (Fig. 6).

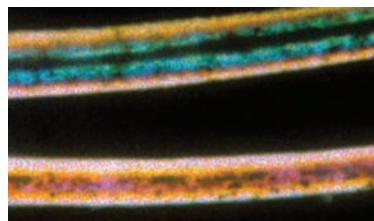


Figure 5 - CHS: hair under polarized light microscopy, bright shafts of different colors (x40).

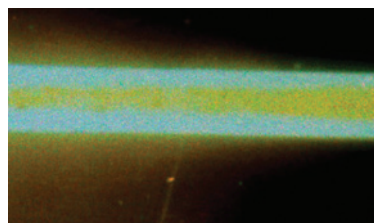


Figure 6 - GPS: hair under polarized light microscopy, whitish hair (x40).

Table 1 - Immunologic data for patients with Chédiak-Higashi syndrome (CHS) or Griscelli-Prunieras syndrome (GPS).

Patient	Case 1	Case 2	Case 3	Case 4
Gender (F, female; M, male)	F	F	M	M
Age (y, years; m, months)	1 y, 8 m	5 y	7 y	4 y
Diagnosis	CHS	CHS	GPS	GPS
IgG mg/dL (normal range)	1919 (331-1164)	1691 (444-1187)	1130 (608-1229)	589 (444-1187)
IgA mg/dL (normal range)	233 (14-105)	197 (25-152)	82 (33-200)	18 (25-152)
IgM mg/dL (normal range)	142 (41-164)	421 (41-186)	289 (47-197)	116 (41-186)
Antibody production	Negative	np	Present	np
NBT test standard/stimulated	53/94% ^a	85/90% ^a	23/28% ^b	np
Chemotaxis standard/stimulated (normal range = 30.2-45.3 μ/91.4-137.2 μ)	30 μ / 68 μ	52 μ/72 μ	np	np
Hb g/dL	10.1	11	8.7	14.4
Ht %	31	32	29	43.7
Leukocytes/mm ³	7400	4800	16300	5400
Neutrophils %	10	27	12	70.9
Lymphocytes %	78	68	88	20.9
Monocytes %	10	2	0	7.3
Platelets/mm ³	580000	270000	215000	294000
Lymphocyte proliferative response to mitogens and candidin	nl	nl	nl	nl
CD3 %	np	64.6	84.9	83.2
CD4 %	np	39.3	50.2	42.1
CD8 %	np	23.9	27.9	43.3

CHS: Chédiak-Higashi syndrome
GPS: Griscelli-Prunieras syndrome
np: not performed
nl: normal

^a stimulation with PMA (phorbol myristate acetate): >95% after stimulation; NBT: nitroblue tetrazolium

^b stimulation with LPS (lipopolysaccharide): > 20% after stimulation

DISCUSSION

The main signs of dermatologic interest in CHS and GPS are generalized skin hypopigmentation at birth with tanning capacity after sun exposure, hairs with a unique silvery sheen, and more rarely, pigmentary dilution of the iris. This phenotype has been described as "partial albinism".^{8,9} Albinism comprises a group of hereditary disorders in which defective genes participate in the melanin synthesis pathway; while the pigmentary alterations in CHS and GPS depend on impaired transference of melanosomes from melanocytes to keratinocytes due to mutations in some genes regulating intracellular organelle transport.^{10,11} Because of these molecular differences between albinism and CHS/GPS, the two latter entities are preferably designated as "silvery hair syndromes".¹⁰⁻¹²

Chédiak-Higashi syndrome (OMIM #214500) is a very rare systemic disorder, inherited as an autosomal recessive trait.¹³ Parental consanguinity is frequently reported in affected patients,^{12,14} but this was only confirmed in case 1 of our series. The defective CHS gene, called CHS1/LYST, maps to chromosome 1q42-43.⁵ It participates in regulation of lysosomal trafficking, and when altered, causes an aberrant fusion of diverse intracellular organelles, giving rise to the abnormally giant granules seen in several tissues that constitutes the hallmark of CHS.^{4,5,9,15,16}

Dermatologic manifestations in CHS range from mild-to-severe pigmentary dilution of skin, hair, and eyes. The fair skin of these patients usually takes a slate-gray hue, as seen in our cases 1 and 2. It appears to be less pigmented when compared to other family members and displays an increased susceptibility to sunburn.¹⁶ Hyperpigmentation of sun-exposed areas, as occurred in both of our CHS patients, has been previously described, but a satisfactory explanation for this rare observation is still absent.^{16,17} An unusual gray or silvery metallic sheen of scalp hair (present in the 2 patients reported here), eyelashes, and eyebrows is reported as the most conspicuous finding.¹³ The iris ranges from blue to brown.¹³ Strabismus, nystagmus, and photophobia are common, but visual acuity is usually normal.^{3,5}

Immunological dysfunction manifests as early, severe, and recurrent infectious episodes; mainly involving the skin, the upper respiratory tract, and the lungs. The most common causative organisms isolated are *Staphylococcus aureus*, *Streptococcus pyogenes* and *S. pneumoniae*.^{3,5,7,15,18-20} The main immunologic defects consist of impaired chemotaxis of granulocytes, together with defective intracellular killing, diminished antibody-dependent cytotoxicity, and defective natural killer cell function.^{4,5,9,13,21} A history of several bacterial pneumonias and chemotaxis in the lower limit were established for case 1.

Patients who live long enough may develop progressive neurological deterioration, resembling spinocerebellar degeneration.^{4,5,22} Near adolescence, most patients enter the so-called "accelerated phase," characterized by pancytopenia, hepatosplenomegaly, and lymphohistiocytic infiltrates into major organs. It has a ruinous prognosis, and death generally occurs from infection or bone marrow failure.^{13,21} Currently, the only curative treatment is bone marrow transplantation.⁹ To date, neither of our patients had developed neurological impairment nor entered the accelerated phase.

Griscelli-Prunieras syndrome (GPS), also known as "partial albinism with immunodeficiency," is an autosomal recessive disorder. Parental consanguinity was established in one of our two cases. The identification of specific genetic defects underlying GPS is the basis of the current classification into 3 types. In both GPS type 1 (GPS1-OMIM #214450), also known (and preferentially designated) as Elejalde syndrome (ES-OMIM #256710), and GPS type 2 (GPS2-OMIM #607624), the mutated genes map to chromosome 15q21.^{17,25} Recently, GPS type 3 (GPS3) has been recognized as being determined by a mutation at a different band (2.37) of the same chromosome 15.²³ Products of the altered genes in GPS participate in melanosome transference from melanocytes to keratinocytes.

All the GPS subgroups share the same phenotype of pigmentary dilution. Classical findings resemble those of CHS, i.e., silvery-gray hair, pale skin with persistent capacity for tanning when sun-exposed, and ocular alterations secondary to pigment diminution.^{7,22,24} In both GPS patients reported here, the most evident dermatologic finding was the presence of silvery hair.

Although having a similar pigmentary phenotype, patients with GPS have different prognoses depending on the subtype. Patients with GPS2 present a history of severe infections caused by immunologic defects (absence of delayed-type cutaneous hypersensitivity and impaired natural killer cell function).²² Hypogammaglobulinemia has also been reported.^{7,22} Interestingly, low levels of both serum IgA and IgG were detected in patient 4. In advanced disease, patients develop an acute phase of uncontrolled lymphocyte and macrophage activation (hemophagocytic disorder). Based on clinical and laboratory findings, we concluded that both of our GPS cases correspond to GPS2. In contrast, patients with GPS1 never evolve to accelerated phases, but present progressive and more severe neurological disturbance that results from the limited expression of MYO5A (the defective gene in GPS1) in the central nervous system.²⁵ Finally, clinical expression for patients with GPS3 will be limited to the pigmentary alterations already described. The prognosis for GPS1 and GPS2 is poor. For patients with GPS2, treatment is the same as for CHS pa-

tients.^{7,22} Currently, there is no specific treatment for GPS1, which always has a fatal outcome.^{22,25} In contrast, the prognosis for patients with GPS3 is good, and these patients do not require any treatment.

For cases in which establishing a differential diagnosis between CHS and GPS is difficult, chromosome analysis detecting the specific causative mutation would be more appropriate. Nevertheless, such evaluation is not easily available, especially in developing countries.

Polarized light microscopy is used to examine doubly refractile materials, such as urate crystals, amyloid, cholesterol esters, silica, and certain foreign bodies.²⁶ Keratin also has doubly refractile properties. Therefore, normal hair shafts can be seen under polarized light microscopy as more or less bright bodies, depending on their baseline hair color.

In our experience, findings from polarized light microscopy obtained when analyzing hairs from patients with CHS or GPS complement data regarding the size and distribution of the hair melanin granules that are found under light microscopic examinations. Polarized light microscopy reveals the whitish, monotonous aspect of hairs of patients with GPS, contrasting clearly with their polychromatic appearance in CHS patients. This may be considered a differential element—not previously reported—for distinguishing between these syndromes (CHS and GPS), especially when data from light microscopic hair examination is inconclusive and/or molecular studies are not available. The reasons for this variation as revealed by polarized light microscopy are not known, and we have not found a convincing explanation.

RESUMO

Valente NYS, Machado MCMR, Boggio P, Alves ACF, Bergonse FN, Casella E, Moraes Vasconcelos D, Grumach AS and Oliveira ZNP. Contribuição do estudo dos cabelos com microscopia de luz polarizada ao diagnóstico diferencial das síndromes de Chédiak-Higashi and Griscelli-Prunieras. Clinics. 2006;61(4):327-32.

OBJETIVO: Estudar e comparar o aspecto dos cabelos de portadores das síndromes de Chédiak-Higashi e Griscelli-Prunieras, tanto na microscopia óptica convencional quanto com luz polarizada.

MÉTODO: Cabelos de dois doentes portadores da síndrome de Chédiak-Higashi e de dois portadores da síndrome de Griscelli-Prunieras foram obtidos e estudados tanto à microscopia convencional quanto com luz polarizada.

RESULTADOS: Na microscopia óptica convencional, os cabelos dos doentes portadores da síndrome de Chédiak-Higashi mostraram grânulos de melanina regulares, com distribuição homogênea e de maior tamanho em comparação aos presentes no cabelo normal. À microscopia de luz polarizada notou-se aspecto brilhante e refringência

policromática. Diferentemente, os cabelos dos doentes portadores da síndrome de Griscelli-Prunieras apresentaram à microscopia convencional, grânulos de melanina irregulares e maiores do que os presentes no cabelo normal e os presentes nos cabelos dos doentes portadores da síndrome de Chédiak-Higashi, preferencialmente próximos à medula das hastes pilosas. À microscopia de luz polarizada apresentaram aspecto monotona e esbranquiçado.

CONCLUSÃO: O exame dos cabelos pela microscopia convencional nas síndromes de Chédiak-Higashi e Griscelli-Prunieras revela diferenças sutis no reconhecimento dessas doenças. No presente trabalho apresentamos evidência de que o exame das hastes pilosas com microscopia de luz polarizada - não descrito previamente - contribui na diferenciação de ambas doenças sugerindo que esse seja um método diagnóstico útil na distinção entre as síndromes de Chédiak-Higashi e Griscelli Prunieras, especialmente nos casos em que estudos moleculares mais sofisticados não estejam disponíveis.

UNITERMOS: Cabelo Prateado; Chédiak-Higashi; Griscelli-Prunieras; Microscopia óptica de luz polarizada.

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