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PROGRESSIVE MACULAR HYPOMELANOSIS (PMH) - TREATABLE BUT OFTEN MISDIAGNOSED - GERMAINE N RELYVELD

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PROGRESSIVE MACULAR HYPOMELANOSIS (PMH) TREATABLE BUT OFTEN MISDIAGNOSED

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	Prof. dr. J. Lambert

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List of abbreviations

AFLP	amplified fragment length polymorphism
bcUVA	benzoyl peroxide 5% hydrogel, clindamycin 1% lotion, UVA
CIE	Commission International de l'Éclairage
DNA	deoxyribonucleic acid
ESR	erythrocyte sedimentation rate
EPA	extensive pityriasis alba
fUVA	fluticasone, UVA
PA	pityriasis alba
PV	pityriasis versicolor
PMH	progressive macular hypomelanosis
P. acnes	*Propionibacterium acnes*
PUVA	psoralen ultra-violet A
rRNA	ribosomal ribonucleic acid
SNIP	Stichting Nederlands Instituut voor Pigmentstoornissen
UVA	ultra-violet A

Contents

GN Relyveld
HE Menke
W Westerhof

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1 GENERAL INTRODUCTION AND AIMS OF THE THESIS

Historical background and terminology

Until recently progressive macular hypomelanosis was an unknown disease entity. The term "progressive macular hypomelanosis" was coined by Guillet *et al*. (1985, 1988, 1992) in the 1980s to describe a pigment disorder in people of mixed racial (Negroid and Caucasoid) ancestry living in France, but originating from the French Caribbean islands. In the Netherlands patients with an identical clinical picture were identified by Menke *et al*. (1989, 1997, 2006) in the mid-1980s. They designated this condition "nummular and confluent hypomelanosis of the trunk".

A similar entity was also reported by several other authors from different parts of the world, with many descriptive names being used to identify it. Borelli (1987) from Venezuela named the condition "cutis trunci variata"; Lesueur *et al*. (1994) from Martinique, West Indies, called it "creole dyschromia"; Fitzpatrick (1996) from the USA used the term "idiopathic multiple large macular hypomelanosis". Recently Di Lernia and Ricci (2005) assumed that PMH and extensive pityriasis alba (EPA), as described by Zaynoun *et al*. (1983, 1986), are the same disease, based on the clinical and histological characteristics of both entities, however, although the clinical characteristics are similar, there are differences in the histological characteristics, with EPA showing histologically eczematous characteristics and PMH only diminished pigment in the epidermis.

Nowadays most authors choose for the term PMH, and until the aetiology of the condition is fully established this can be considered a plausible choice.

Epidemiology

Although the true prevalence of PMH is unknown, it seems to be a common disorder. In 2005 at The Netherlands Institute for Pigment Disorders (SNIP) 48 patients were diagnosed with PMH, compared to 23 patients with pityriasis versicolor and 17 patients with pityriasis alba. The number of PMH patients seen each year at the SNIP has been growing since. Lesueur *et al*. (1994) identified 121 patients with PMH during a systematic screening of 511 patients for leprosy in Martinique. In 2006 Kumarasinghe *et al*. (2006) mentioned that PMH was a common skin disorder in Singapore, although the exact number of patients was not given. PMH is probably more often diagnosed in countries with a population with darker skin types for the obvious reason that white spots are more easily recognized in pigmented skin. Contact with colleagues from Ivory Coast, Colombia, Brazil, the West Indies, India,

Sri Lanka, Singapore, Indonesia and The Philippines indicates that this disorder is most likely prevalent in many parts of the world.

At the SNIP women seem to be predominantly affected with PMH. Guillet *et al*.(1985, 1988, 1992) observed PMH mainly in women, but Borelli (1987) mentioned an equal distribution in both sexes. PMH is mostly observed in adolescents and young adults (Menke *et al*. 1997, Lesueur *et al*. 1994, Fitzpatrick 1996). Guillet *et al*. (1992), Borelli (1987) and Fitzpatrick (1996) recognized PMH only in racially mixed people, but at the SNIP we have seen the disorder in a variety of patients of mixed and "unmixed" ethnicities. Kumarasinghe *et al*. (2006) observed PMH in Chinese, Mongoloid and Indian people.

Histology and electron microscopy

Guillet *et al*. (1992) as well as Kumarasinghe *et al*. (2006) performed histological examinations on the skin of PMH patients. Hematoxylin-eosin staining of the skin of hypopigmented spots showed only a subtle decrease in melanin content in the epidermis compared with that in normal adjacent skin. There was no significant inflammatory infiltrate or epidermotropism of leukocytes. Fontana-Masson-staining showed overall reduction in melanisation of the basal cell layer of lesional skin. Melanocytes stained negative for HMB 45 and Melan A stains. S100 staining did not detect any differences in the number of melanocytes, Langerhans cells, or other dermal dendritic cells. Occasional melanophages were noted in the dermis of both lesional and normal skin on hematoxylin-eosin and CD68 staining.

Etiology and Pathogenesis

Borelli (1987) considered PMH to be a genodermatosis, based on the fact that he observed the disorder in family members. According to Guillet *et al*. (1992) correlation of clinical and electron microscopic findings led to the hypothesis that the gene pair partly determining the Negroid type of melanin production in mixed races could be inactivated, at least temporarily, under several unknown conditions, thereby producing a switch from Negroid to Caucasoid melanogenesis. Fitzpatrick (1996) suggested that the hypopigmentation of PMH might originate from a fungal infection, with the pigment changes remaining long after the infection had disappeared.

In 2001 Westerhof observed a red follicular fluorescence in the hypopigmented spots that was not present in normal adjacent skin, while examining patients with PMH under strictly blinded conditions in a dark room using a Wood's lamp. This gave rise to further investigations.

Clinical characteristics

PMH is a morphologic entity characterized by ill-defined nummular hypopigmented macules, symmetrically localized predominantly on the trunk, but sometimes progressing to the neck and the face and the proximal parts of the extremities. In the majority of patients, a rather well defined hypopigmented area that appears to originate from a confluence of macules can be recognized on the front and back of the trunk. The width of this confluent region varies from patient to patient. In the

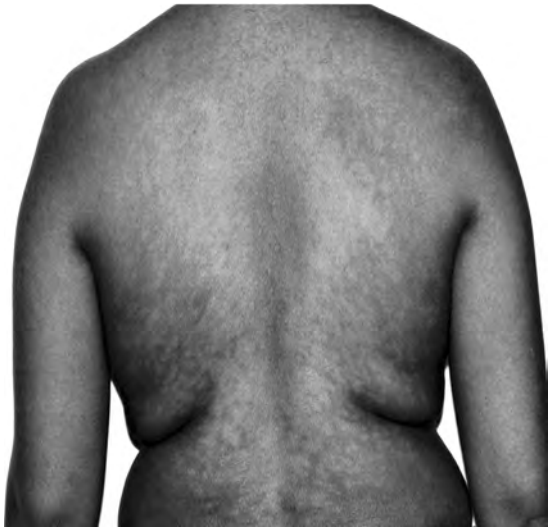

Figure 1 28 year old female with PMH.

lateral regions of the trunk, more or less round solitary macules can be recognized (Figure 1). Kumarasinghe *et al*. (2006) also observed the macules on the buttocks and thighs. There seems to be no history of pruritus, pain, or a preceding inflammatory dermatosis.

The clinical descriptions provided by Guillet *et al*. (1992), Menke *et al*. (1997), Borelli (1987), Lesueur *et al*. (1994) and Kumarasinghe *et al*. (2006) correspond to a large

extent with our observations. However, Guillet *et al*. (1992) have stated that the macules were absent in the dorsolumbar line, something we could not confirm. Additional laboratory tests (ESR, complete blood count, blood glucose, liver enzymes and urea) and urine analysis were within normal limits and potassium-hydroxide tests of skin scrapings were negative (Menke *et al*. 1997), Kumarasinghe *et al*. 2006). Guillet *et al*. (1992) ruled out leprosy as a cause after performing physical examination and obtaining a skin biopsy. Furthermore Guillet *et al*. (1992) and Menke *et al*. (1997) reported negative serology tests for syphilis.

Clinical implications for patients

In the Netherlands PMH is mainly a cosmetically disturbing disorder. However, especially in tropical countries were leprosy is endemic, patients may worry about the probability of having leprosy when they see the hypopigmented spots on their skin.

Differential Diagnosis

PMH should be distinguished from other disorders with acquired hypopigmentation appearing only or mainly on the trunk. Such disorders can be divided into four groups:

1. Hypomelanosis caused by non-bacterial / non-fungal inflammatory skin disorders, i.e. pityriasis alba and post inflammatory hypopigmentation (e.g. after atopic dermatitis, contact dermatitis and psoriasis).
2. Hypomelanosis caused by leprosy, i.e. hypopigmented macules in borderline tuberculoid leprosy or borderline lepromatous leprosy.
3. Hypomelanosis caused by fungi and yeasts, i.e. pityriasis versicolor and seborrhoic dermatitis.
4. Hypomelanosis caused by proliferative neoplastic disorders, i.e. hypopigmentation in cutaneous T-cell lymphoma (mycosis fungoides).

Table I summarizes the important differences between these skin disorders.

Treatment

Most authors who described PMH did not mention any effective treatment. Menke *et al*. (1997) attempted several treatment modalities, including topical and systemic antifungal agents and topical corticosteroids, but none of these treatments were successful. They observed disappearance of the hypopigmented spots with

psoralen plus UVA (PUVA) therapy; however, after cessation of this treatment, the induced pigmentation disappeared which resulted in exactly the same pattern of hypopigmentation as before.

Prognosis

Guillet *et al*. (1992) suggested that PMH disappeared spontaneously within 3-5 years. Menke *et al*. (1997) could not confirm this spontaneous regression; on the contrary, the disorder appeared to be stable during a follow-up period of 10 years, and spontaneous repigmentation was never seen. Lesueur *et al*. (1994) found the disorder to be persistent for more than 25 years. The fact that it has never been observed in elderly people indicates a spontaneous disappearance after young adulthood.

Table I Differential diagnosis of progressive macular hypomelanosis

Disorder	Clinical features
Progressive macular hypomelanosis	Symmetric, hypopigmented, ill defined spots mainly on the trunk, sometimes on the proximal extremities; non scaling, non itchy
Pityriasis versicolor (Roberts 1969, Crespo and Delgado 2002, Morishita and Sei 2006, Prohic and Ozegovic 2007, Hay and Moore 2004)	Irregular hypopigmented, sharply defined, often asymmetric spots; mottled distribution; scaling; mostly on neck and trunk, sometimes elsewhere
Pityriasis alba (Lin and Janniger 2005, Blessmann *et al*. 2002, Vargos-Ocampo 1993, Hurwitz 2006, Holden and Berth-Jones 2004)	Oval or irregular hypopigmented plaques; well defined border; fine lamellar scaling; initially erythematous; predilection for face, sometimes neck upper trunk and proximal extremities; usually disappearing in a few months or changing place; may be pruritic
Borderline tuberculoid leprosy, Borderline lepromatous leprosy (Ridley and Jopling 1966, Pardillo *et al*. 2007, Britton and Lockwood 2004, Yawalkar 2002, Naafs and Faber 2006)	Hypopigmented macules with varying symmetry; remaining in exactly the same place; few to multiple lesions; marked to mild loss of sensation to fine touch
Mycosis fungoides (Smith and Samman 1978, Lambroza *et al*. 1995, Nickoloff 1988, Shapiro and Pinto 1994, Whittaker and MacKie 2004)	Circular or oval areas of hypopigmentation with well or ill defined borders on trunk and extremities, rarely the face; often symmetric; pruritus is variable
Post inflammatory hypopigmentation	positive history of former skin disorders; restricted to sites of primary lesion;

Aims of the thesis

PMH is a common skin disorder occurring worldwide. In our experience it has frequently been disregarded or misdiagnosed and consequently mistreated. It is often considered a postinflammatory hypopigmentation after either pityriasis versicolor or an inflammatory dermatosis such as atopic dermatitis. There is still much confusion whether PMH is a separate entity or part of (or a remainder of) an existing (hypopigmented) skin disorder. To gain a better insight in this skin disorder we conducted the following studies.

We noticed that in patients with PMH a red, follicular fluorescence can be observed in lesional skin, which is absent in the adjacent normal skin, when the skin is illuminated

Histology of hypopigmented lesions	Additional findings
Loss of epidermal pigment, no dermal abnormalities	Red follicular fluorescence in the white spots under Wood's light in a dark room
Hypopigmentation of the epidermis, spores and hyphae in the upper layers of the epidermis; slight inflammation in the dermis	Positive potassium hydroxide preparations; cultures show Malassezia species
Discrete eczematous changes in the epidermis and dermis with mild spongiosis, hyperkeratosis and patchy parakeratosis	Sometimes positive tests for atopy
Granulomatous reaction with varying composition of mononuclear phagocytes and lymphocytes; presence of M. Leprae depending on position in the leprosy spectrum	
Pautrier micro-abcesses, epidermotropism of lymphocytes, mild superficial perivascular infiltrate of lymphocytes	
Reduction in melanin pigment in basal layer; sometimes pigment-containing melanophages present in upper dermis; residual features of inflammatory dermatosis present	

with a Wood's lamp in a dark room. This ignited the idea that this could possibly be the result of porphyrin producing microorganisms like *Propionibacterium acnes (P. acnes)*, which are known to show red follicular fluorescence under UV irradiation (or Wood's light) (Johnsson *et al*. 1987). The observation of this fluorescence in lesional skin of PMH patients gave rise to the question if there might be a relation between bacteria producing this fluorescence and the hypopigmented macules.

Chapter two describes a study which compares conventional culture results from biopsy swabs taken from lesional follicular and inter-follicular skin in PMH patients as well as biopsy swabs taken from normal follicular and inter-follicular skin in the same patients.

The red follicular fluorescence that is observed in lesional skin of PMH patients is comparable with the red fluorescence seen in acne. In acne it is caused by *P. acnes* bacteria residing in the pilosebaceous ducts of the skin. These bacteria are known for their contribution to the development of acne. Since previous studies confirmed the presence of *P. acnes* solely in lesional skin of PMH it made us wonder why acne is hardly ever observed in PMH patients. Therefore we conducted a study to further identify and compare the precise microbial species related to acne and PMH.

Chapter three describes a study in which DNA fingerprinting by Amplified Fragment Length Polymorphism (AFLP), 16S rRNA gene sequencing and biochemical identification were performed to identify the exact bacterial species that is related to PMH but most probably can not be discriminated by conventional culture methods from the bacterial species causing acne. In short AFLP is a genetic mapping technique that uses specific amplification of a subset of restriction enzyme digested DNA fragments to generate a unique fingerprint for a particular genome. The AFLP has been widely applied in the identification and genotyping of various organisms because of its high discriminatory power and reproducibility, including *Propionibacteria* (Vos *et al*. 1995, Savelkoul *et al*. 1999, Mohammadi *et al*. 2005). 16S DNA sequencing is the golden standard for taxonomic species identification; the comparison of the 16S rRNA gene sequences allows differentiation between organisms at the genus level in addition to classifying strains at multiple levels, including the species and subspecies level (Clarridge 2004).

Various descriptions have been given of PMH the last twenty years. All were more or less comparable, but none were systematically obtained and there was no consensus with regard to the clinical characteristics and natural course of the disorder. In order to gain a clear view, we developed a questionnaire for a survey among PMH patients at our institute.

Chapter four presents the results of this questionnaire. To provide a thorough and convenient arrangement of the natural course and clinical characteristics of PMH we additionally compared our results with previous descriptions from the literature.

Previous studies provided us with little information concerning the pathogenesis of PMH. We do know that there is a decrease in epidermal melanin in lesional skin of PMH and that electron microscopy studies thus far showed less mature melanosomes in lesional skin. But the question remains why these changes occur and what actually happens in the melanocytes and keratinocytes.

Chapter five further explores this question. To gain more insight into the mechanisms involved in the alterations in pigmentation in PMH an electron microscopic study was conducted in which biopsies taken from lesional skin were compared with biopsies taken from adjacent non-lesional skin of PMH patients. Electron microscopy provides us with a much higher magnification than light microscopy, making it possible to assess the skin at melanosomal level and therefore making it possible to view melanocytes with their melanosomal precursors, transfer of melanosomes to keratinocytes and melanosomes in the keratinocytes.

The following (logical) question that arose was that if *P. acnes* bacteria are indeed the causative agents in PMH, would antibacterial therapy be effective in the treatment of PMH and is there any difference in treatment results between an antibacterial approach and an anti-inflammatory approach?
The last study in **Chapter six** describes the results of a within-patient, left-right comparison of 5% benzoyl peroxide hydrogel/1% clindamycin lotion in combination with UVA irradiation (antibacterial treatment plus stimulation of pigmentation) versus 0.05% fluticasone propionate cream in combination with UVA irradiation (a typical and effective modality of anti-inflammatory treatment) in patients with PMH.

Chapter seven has been included in the thesis to provide an example of the existing confusion, when diagnosing skin diseases that resemble PMH.

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Propionibacterium acnes and the pathogenesis of progressive macular hypomelanosis. *Arch Dermatol*. 2004;140:210-214

2

PROPIONIBACTERIUM ACNES AND THE PATHOGENESIS OF PROGRESSIVE MACULAR HYPOMELANOSIS

ABSTRACT

Introduction: Progressive macular hypomelanosis is a common hypopigmentation mainly on the central parts of the trunk, predominantly in young adults, especially women. It is often mistaken for pityriasis versicolor and pityriasis alba. It occurs in all races and has been described in many parts of the world. We discovered follicular red fluorescence restricted to lesional skin. We suspected a relation with a porphyrin-producing bacteria residing in sebum of the pilosebaceous duct, and we therefore performed a study in 8 patients.

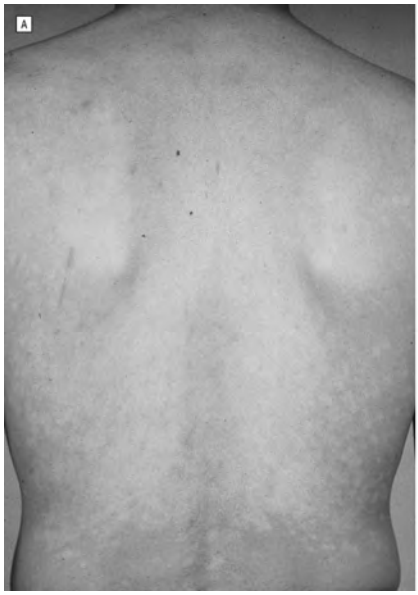
Results: In all biopsy specimens taken from lesional skin of 8 women, we could demonstrate gram-positive bacteria in the pilosebaceous duct, and a mild perifollicular lymphocytic infiltrate was seen. In all but 1 patient, *Propionibacterium acnes* was yielded from cultured biopsy specimens taken from follicular lesional skin. Healthy follicular skin did not show bacteria in histological sections, and cultures did not yield anaerobic bacteria.

Conclusions: There seems to be a relation between the presence of *P. acnes* and the hypopigmented macules. We propose that a factor is produced by these strains of *P. acnes,* which interfere with melanogenesis. Based on these observations, we are undertaking a clinical trial to find a treatment for this troubling, intractable disease.

INTRODUCTION

Progressive macular hypomelanosis (PMH) of the trunk is characterized by ill-defined nummular, hypopigmented, nonscaly macules on the front and back of the trunk, with confluence of the macules in and around the midline (Figure 1A,B). It occurs in young adults of both sexes, but primarily women. Progressive macular hypomelanosis of the trunk is described by Guillet *et al*. (1988, 1992), in people of mixed racial (white and black) ancestry originating from the French Caribbean. This entity was also described or at least recognized independently by several investigators from different parts of the world, using the following variety of descriptive terms: *cutis trunci variata* in Venezuela (Borelli 1987), *Creole dyschromia* in the French West Indies (Lesueur *et al*. 1994), *idiopathic multiple large-macule hypomelanosis* in the United States (Sober, Fitzpatrick, 1996), and *nummular and confluent hypomelanosis of the trunk* by our group in the Netherlands (Menke *et al*. 1989, 1997, 1998). From comparison of the clinical descriptions, it seems reasonable to assume that they all refer to the same pigmentary disorder.

The pathogenesis of PMH of the trunk is unknown, although several hypotheses have been offered. According to Guillet *et al*. (1988), this disorder is caused by a "melting" of genes of white and black parents. This view is based on the fact that the disorder



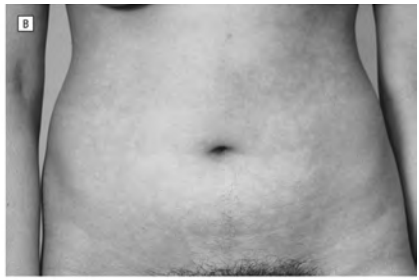


Figure 1A Progressive macular hypomelanosis in a male patient of skin type V, with a typical distribution of confluent hypopigmented macules on the back.

Figure 1B Progressive macular hypomelanosis in a female patient of skin type V with hypopigmented lesions on the abdomen.

is seen in people of mixed racial origin and furthermore on the ultrastructural finding of stage IV single melanosomes (in black skin) in healthy-looking skin and small stages I through III aggregated melanosomes (in white skin) in the hypopigmented spots. However, our observation that the disorder also occurs in people of other racial origin, including "pure" white patients, is an argument against this racial theory (Menke *et al*. 1997). Moreover, our own electron microscopic investigations do not confirm these findings (GN Relyveld, KP Dingemans, W Westerhof, unpublished data, May 2002). Borelli (1987) suggests that this is a genodermatosis, but hard facts in support of this idea are lacking in his article. A hereditary factor, as in many other disorders, might indeed play a role; additional research is needed to clarify this point. In a comment on a publication by Lesueur *et al*. (1994), Sober (1996) and FitzPatrick (1996) state that this disorder has been seen in African Americans in Boston, Massachusetts, as well as in the West Indies, and they think that it might be related to tinea versicolor. However, the causative organism of tinea versicolor, the yeast *Malasezia furfur* (also known as *Pityrosporum orbiculare / ovale*), was never identified by us (and others) in skin lesions of this disorder, and we have never seen a case of PMH of the trunk that evolved from a preceding tinea versicolor. We also could not relate it to atopy as in pityriasis alba or to contact dermatitis, psoriasis, or seborrheic eczema, which cause postinflammatory hypopigmentation. Other hypomelanoses due to microorganisms (eg, leprosy or pinta) were excluded (Menke *et al*. 1998).

We propose a new hypothesis on the pathogenesis of PMH of the trunk. We noticed that patients with PMH of the skin show pointed red fluorescence in a follicular fashion inside the hypopigmented spots, when observed under Wood's light (Figure 2). The number of fluorescescing follicles is generally higher on the lateral side of the

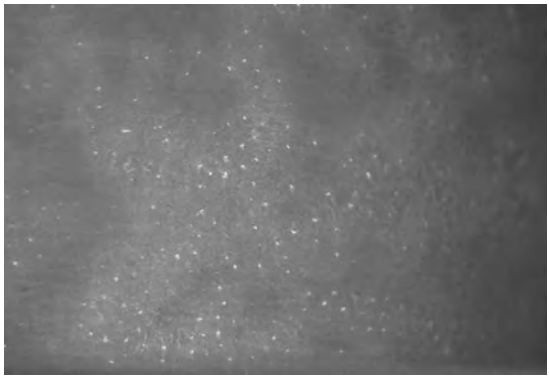


Figure 2 Red fluorescence in a follicular pattern, restricted to lesional skin.

trunk, where isolated nummular hypopigmented spots are present; here probably the hypopigmentation is slowly expanding. Usually the healthy pigmented skin of the trunk does not show any fluorescence. However, in the healthy-looking skin close to the rim of the hypopigmented macules, a follicular fluorescence can sometimes be seen. On meticulous inspection, a nearby, often small, probably incipient hypopigmented spot, only a few millimeters in diameter around that fluorescing follicle, can be seen. Based on these observations, we now hypothesize that there might be a relation between the red fluorescing follicles and the hypopigmented macules.

Dermatologists are familiar with the phenomenon of red follicular fluorescence, which is caused by the presence of *Corynebacterium* species or *Propionibacterium acnes,* producing porphyrins that are responsible for the fluorescence induced by UV radiation as under Wood's light (Bommer 1927). We therefore investigated 8 patients for the presence of *P. acnes* in lesional skin.

METHODS

Patients

We included 8 patients with PMH in this study to investigate the possible relation between the red fluorescing follicles (*P. acnes*) and the hypopigmentation. All were women aged 18 to 38 years (mean age, 29 years). All 8 patients had ill-defined nummular, hypopigmented, nonscaly macules on the front and back of the trunk, with confluence of the macules in and around the midline. Participants were from different ethnic backgrounds. They originated from the Netherlands (skin type III), Turkey or Morocco (skin type IV), and Suriname (Hindustani or Creole people with skin type V) (Table I). They all gave their informed consent to this study.

Histological investigation

We obtained 2-mm biopsy specimens from the lesional follicular skin, healthy follicular skin, and inter-follicular lesional and healthy skin. Hematoxylin-eosin, gram, and periodic acid–Schiff staining were performed. A gram stain finding is one of the cornerstones for bacterial identification, but it also serves as a useful technique for rapid detection of microorganisms in clinical samples.

Table I Demographic Data on the Progressive Macular Hypomelanosis Patients in this Study

Patient No.	Skin type	Sex	Age, y	Site of Lesions	Duration, y
1	III	F	28	B, A, F	11
2	III	F	38	B, A	Unknown
3	III	F	25	B, A	5
4	III	F	37	A	4
5	IV	F	31	B, UA	Unknown
6	V	F	27	A	Unknown
7	V	F	18	B	1.5
8	IV	F	28	B	5

Abbreviations: A, Anterior side of the trunk; B, back of the trunk; F, face; UA, upper arms.

MICROBIOLOGICAL INVESTIGATION

Sampling and Culture Conditions

The skin was first disinfected with 70% alcohol, without other antibacterial agents, to eliminate superficial skin flora. After the skin dried, 2-mm biopsy specimens were obtained from lesional skin containing a fluorescent hair follicle and from nonlesional skin containing a nonfluorescent hair follicle.

We also obtained biopsy specimens from inter-follicular healthy and lesional skin. For microbiological culture, all biopsy specimens were cut transversally, and the dissected sides were immediately swapped on blood-culture agar plates (Colistin Nalidixic Agar; Becton, Dickinson and Company, Franklin Lakes, NJ) with thioglycolate-enriched broth. One half was cultured under aerobic conditions (5% carbon dioxide at 37°C), and the other half was cultured under anaerobic conditions (80% nitrogen/10% hydrogen/10% carbon dioxide) for 48 hours at 37°C.

Identification

Anaerobic colonies were subcultured under aerobic and anaerobic conditions for 48 hours. The anaerobic colonies underwent gram staining. The gram-positive rods were identified with a commercial identification kit (Rapid ID 32A; bioMérieux Vitek Inc, Lyon, France).

Propionibacterium acnes is a gram-positive, non–spore-forming, anaerobic bacteria. Bacteria were identified per morphologic colony type.

ANTIBIOTIC SENSITIVITY OF *P. ACNES* ISOLATES

An antimicrobial sensitivity test was performed with the disk diffusion method with agar plates supplemented with 5% sheep's blood. The plates were incubated at 37°C for 24 to 48 hours under anaerobic conditions. The antimicrobial agents used in this study included penicillin, amoxicillin, amoxicillin-clavulanate combination, piperacillin-tazobactam combination, erythromycin, clindamycin, and metronidazole.

RESULTS

Histological findings

Histological examination of the hypopigmented lesions revealed only a decrease of melanin content in the epidermis compared with the adjacent healthy skin; there were no abnormalities in the dermis. There were no signs of eczema as in pityriasis alba, seborrheic eczema, or psoriasis. In the lesional skin of all patients, there was sometimes a mild perifolliculitis (Figure 3A). In the stratum corneum, no spores, hyphae, or bacteria were seen. In the specimens stained with periodic acid–Schiff, no spores or hyphae were found in middle portion of pilosebaceous duct; however, they contained a pure population of gram-positive bacteria (Figure 3B), which showed a rodlike structure with arborizing growth pattern that was consistent with features of *P. acnes*. The findings were also positive for periodic acid–Schiff reaction but not for acid-fast staining.

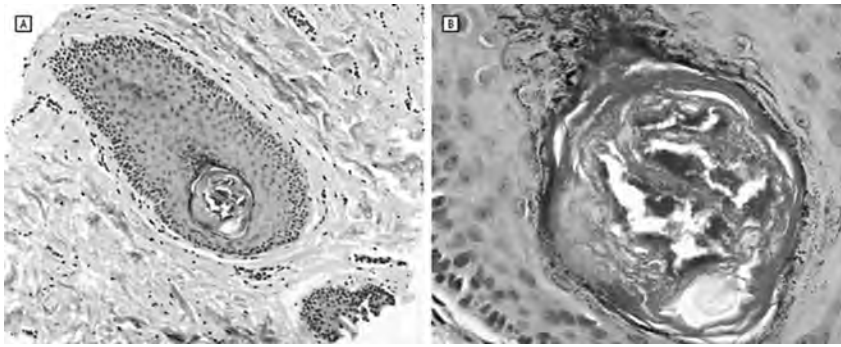


Figure 3A Hematoxylin-eosin staining of follicular lesional skin shows a mild perifollicular infiltrate of lymphocytes (original magnification x100). B Gram staining of a cross section of a pilosebaceous duct with a high population density of microorganisms, which on culture yield appear to be *Propionibacterium acnes* (original magnification x400).

Table II Culture of Biopsy Specimens for Propionibacterium acnes Sensitivity/Resistance

Patient No.	Lesional Skin	Nonlesional Skin	Penicillin	Amoxicillin	Amoxicillin-Clavulanate	Piperacillin-Tazobactam
1	+	-	S	S	S	S
2	+	-	S	S	S	S
3	+	-	S	S	S	S
4	+	-	S	S	S	S
5	+	-	S	S	S	S
6	+	-	S	S	S	S
7	-	-	-	-	-	-
8	+	+	S	S	S	S

Abbreviations: R, resistant; S, sensitive; -, negative; +, positive.

Microbiological findings

The cultures from inter-follicular skin of lesional and healthy skin of patients with PMH were negative for *P. acnes*. In biopsy specimens from lesional follicular skin of all patients, *P. acnes* could be isolated except in patient no.7. The histological picture of the hair follicles in her lesional skin, however, demonstrated gram-positive bacteria typical of *P. acnes.* Patient no.8 showed a positive culture yield in clinically normal skin (Table II). This biopsy specimen was taken from a close distance to the lesional skin. No *P. acnes* was found in cultures of nonfollicular lesional and healthy skin.

Propionibacterium acnes isolates demonstrated high-level in vitro sensitivity to penicillin, amoxicillin, amoxicillin-clavulanate, piperacillin-tazobactam, erythromycin, and clindamycin and resistance to metronidazole (Table II).

DISCUSSION

We observed red fluorescence in a follicular pattern inside lesional skin of patients with PMH, which coincides with the presence of *P. acnes* yielded from cultures of lesional skin follicles. They could not be retrieved from healthy skin.

The phenomenon of red punctate fluorescence, using examination of facial skin under Wood's light, was first described by Bommer in 1927. The foci of light correspond to porphyrin produced by *P. acnes,* which resides within the hair follicle unit. Using photographic methods, the fluorescence can be demonstrated on film (Burkhart 2001). McGinley *et al*. (1980) revealed that a density of 1000 *P. acnes*

Erythromycin	Clindamycin	Metronidazole
S	S	R
S	S	R
S	S	R
S	S	R
S	S	R
S	S	R
-	-	-
S	S	R

organisms was required for follicular fluorescence to occur, and that the intensity was proportional to the numbers obtained by bacteriologic culture of *P. acnes.*

Propionibacterium acnes is a major inhabitant of adult human skin, and high population densities are associated with skin sites possessing high numbers of sebum-excreting sebaceous follicles (McGinley *et al*. 1978). It is generally considered that this organism has a low virulence in humans. The population densities of these bacteria are low in children, who have low sebum excretion rates (Leyden *et al*. 1975). *Propionibacterium acnes* colonizes the infra-infundibular portion of follicles of sebaceous glands. Sebaceous follicles are most common in the acne-prone areas, such as the cheeks, nose, and forehead, and the midline of the chest and back (Toyoda and Morohashi 2001). This organism is not pathogenic by normal standards because in case of acne, there is minimal correlation between the number of bacteria and the severity and type of acne.

We did not notice acne lesions on the back, the chest, or the face in any of our patients. They also had no history of acne. Although for acne, Koch's famous postulates for the definition of infection have not been met, and we believe in a specific role of *P. acnes* in the promotion of hypopigmentation in PMH. At the periphery of the confluent lesions, small, round hypopigmented macules arise. The fluorescent follicle is always at the center of the lesion, suggesting the diffusion of a hypopigmenting factor migrating from the follicular orifice.

It is possible that *P. acnes* produces a depigmenting agent or a factor that interferes with the melanogenesis in the skin, resulting in hypopigmented spots. The type of

mechanism we now propose is not new in the biology of pigmentary disorders. In 1986, Nazzaro-Porro *et al*. (1986) suggested that the hypopigmentation in pityriasis versicolor is probably due to toxic lipoperoxides formed by the action of *Pityrosporon ovale* on the unsaturated lipids of the skin surface.

Previously, we investigated 50 patients with skin types III, IV, and V and the typical distribution of ill-defined nummular and confluent non-scaly hypopigmented lesions around the midline of the trunk. They were examined for follicular fluorescence in lesional and healthy skin and compared, for the presence of this fluorescence, with 10 patients with pityriasis versicolor and 5 patients with pityriasis alba (W Westerhof, HE Menke, unpublished data, 1999). To observe the red follicular fluorescence, a completely dark room is essential, with the use of a strong Wood's lamp. The fluorescent tubes need to warm up, and the observer has to adapt to the dark environment for at least 3 minutes.

In all 50 patients with clinical signs of PMH, we saw red follicular fluorescence restricted to lesional skin. In the patients with pityriasis versicolor and pityriasis alba, no red fluorescence could be discerned in lesional and normal skin.

No effective treatment of PMH is available at present. Topical and systemic antifungal treatment and topical steroids are ineffective. We never observed spontaneous regression of the lesions; on the contrary, the disorder appeared to be stable or showed a slow progression over time in about 200 patients followed up by us for more than 10 years. With phototherapy or after extensive sun exposure, the white spots can disappear or become less apparent. However, a couple of weeks or months after cessation of this treatment, the induced repigmentation fades away and the hypopigmented spots reappear at exactly the same sites. We are presently undertaking a clinical trial to test a treatment regimen that is directed against the *P. acnes,* while stimulating melanogenesis (GN Relyveld, M Kingswijk, JB Reitsma, W Westerhof, unpublished data, starting in August 2002).

GN Relyveld
W Westerhof
JHC Woudenberg
M Kingswijk
ML Langenberg
CMJE Vandenbroucke-Grauls
PHM Savelkoul

Progressive macular hypomelanosis is associated with a putative new *Propionibacterium* species. Submitted to *Journal of Investigative Dermatology*

3

PROGRESSIVE MACULAR HYPOMELANOSIS IS ASSOCIATED WITH A PUTATIVE NEW *PROPIONIBACTERIUM* SPECIES

ABSTRACT

Introduction*: Propionibacterium acnes* (*P. acnes*) plays an important role in the pathogenesis of acne and progressive macular hypomelanosis (PMH). However, acne lesions are absent in most PMH patients.

Patients and Methods: We characterized bacterial isolates obtained from PMH and acne patients to detect a possible difference.

Amplified Fragment Length Polymorphism of pure cultures from skin swabs of 14 PMH and 10 acne patients resulted in 3 different DNA groups.

Results: Compared to the *P. acnes* reference strain, isolates from group 1 (8 acne and 6 PMH patients) showed a similarity between 55 and 100% suggesting the same species, isolates from group 2 (2 acne patients) showed a similarity between 30 and 55% suggesting a variant of *P. acnes* and group 3 isolates (8 PMH patients) formed a clear distinct DNA group with a similarity of less than 30%. This low level of homology suggested that these isolates belong to a different species. *16S rRNA* gene sequencing and biochemical tests showed minimal differences between the three DNA groups, suggesting a subspecies of *P. acnes.*

Conclusions: The results show a correlation between the presence of group 3 strains and PMH, since these strains were exclusively found in PMH patients. Further research is needed to confirm this relationship.

INTRODUCTION

Progressive macular hypomelanosis (PMH) is a skin disorder characterized by ill defined, nummular, symmetrically localized hypopigmented macules on sebum-rich areas of the skin of young adults, rarely extending to the head and proximal extremities (Figure 1) (Relyveld *et al*. unpublished data 2007). PMH has often been (mis)diagnosed as pityriasis alba (PA) or pityriasis versicolor (PV) even though there are distinct histological and clinical differences (Relyveld *et al*. 2008).

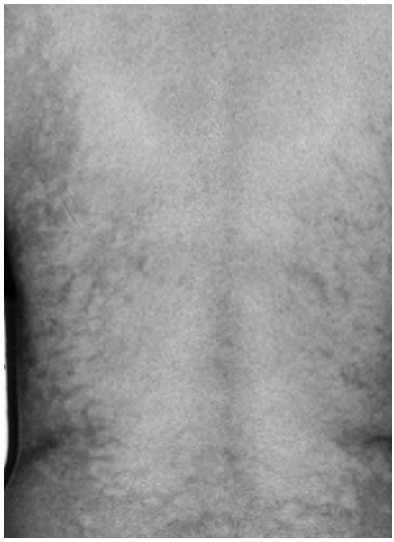


Figure 1 28 year old PMH patient.

In 2004 Westerhof *et al.* hypothesized that PMH might be caused by *Propionibacterium acnes* (*P. acnes*) bacteria. This was based on the observation of red follicular fluorescence in lesional skin of PMH patients, when illuminating the skin with a Wood's lamp in a dark room. This fluorescence was absent in the adjacent normal skin as well as in patients with proven PA (by biopsy) or PV (by KOH tests). Red follicular fluorescence is characteristic for *P. acnes* bacteria residing in pilosebaceous ducts of normal skin and especially in acne prone skin. *P. acnes* is considered to be commensal flora, and accounts for approximately half of the total skin microbiota (Tancrede *et al*. 1992). In PMH patients the red follicular fluorescence is only present in the hypopigmented macules and not in the adjacent normal sebum-rich skin (Westerhof *et al*. 2004).

Conventional cultures from follicles of lesional skin in PMH patients showed *P. acnes* bacteria that could not be cultured from adjacent normal skin. Furthermore the bacteria showed high-level sensitivity to penicillin, amoxicillin, amoxicillin-clavulanate, piperacillin-tazobactam, erythromycin and clindamycin. Resistance was observed for metronidazole (Westerhof *et al*. 2004). These phenotypic characteristics are typical for *P. acnes*.

Since *P. acnes* is particularly implicated as a cause of acne our group conducted a study in 2006 (Relyveld *et al*. 2006) in which we compared anti-acne therapy with anti-inflammatory therapy in the treatment of PMH. Anti-acne therapy had

significantly better treatment results than anti-inflammatory therapy. Our previous findings gave a scientific basis to our hypothesis that *P. acnes* might be the cause of PMH. However, it is notable that acne does not seem to predispose for PMH and patients with PMH do not have acne more often than the general population.[1]

In 1972 two phenotypes of *P. acnes* have been described (Johnson *et al*. 1972), which have been proven to be distinct phylogenetical groups (McDowell *et al*. 2005) called type I and type II *P. acnes*, but the clinical importance of these two types is still unknown. Therefore we hypothesized that the causative bacteria in PMH might be a subspecies of *P. acnes* that can not be differentiated by conventional culturing methods. We decided to further identify the bacteria we cultured from PMH patients and acne patients by molecular strain comparison.

Characterization by the DNA fingerprinting method Amplified Fragment Length Polymorphism (AFLP) has proven to be superior for identification and typing of bacteria at the strain level, compared to identification by conventional culture (Savelkoul *et al*. 1999). AFLP is a genetic mapping technique that uses specific amplification of a subset of restriction enzyme digested DNA fragments to generate a unique fingerprint of a particular genome. AFLP has been widely applied in the identification and genotyping of various organisms, including *Propionibacteria,* because of its high discriminatory power and reproducibility (Savelkoul *et al*. 1999, Vos *et al*. 1995, Mohammadi *et al*. 2005).

16S DNA sequencing is the golden standard for taxonomic species identification; the comparison of the *16S rRNA* gene sequences allows differentiation between organisms at the genus level in addition to classification of strains at the species and subspecies level (Clarridge 2004). The *16S rRNA* gene sequence is composed of both variable and conserved regions. The gene is large enough, with sufficient interspecies-specific polymorphisms to provide distinguishing and statistically valid measurements.

The purpose of this study was to identify, through Amplified Fragment Length Polymorphism (AFLP), *16S rRNA* gene sequencing and biochemical characterization, the bacterial species that is related to PMH. We hypothesized that this species would be different from the bacterial species causing acne, and that it cannot be differentiated from the species that causes acne by conventional culture and biochemical methods. Identification of the bacterial species that causes PMH might lead to better understanding of the disease and better treatment modalities.

MATERIALS AND METHODS

Patients and inclusion criteria

Fourteen patients with PMH and 10 patients with acne were included in this prospective study. All patients were seen at the Netherlands Institute for Pigment Disorders, Amsterdam, the Netherlands, a tertiary referral center for pigment disorders. The Declaration of Helsinki protocols were followed and the study was approved by the medical ethical commission of the Academic Medical Center in Amsterdam, the Netherlands. All patients provided written informed consent before entering the study. Patients under the age of 18 needed parental consent to be included.

The diagnosis of PMH was based on a combination of clinical signs (symmetrically distributed, ill-defined nummular hypopigmented macules, especially on the trunk), the presence of red follicular fluorescence in the lesional skin when examined with a Wood's lamp in a dark room and negative KOH tests. Patients with any form of acne on the trunk (mild, moderate, severe) and without hypopigmented lesions on the skin were included in the acne group. Patients were excluded from the study if they had a history of atopic dermatitis, seborrheic dermatitis and psoriasis and/or if they were allergic to the anaesthetic used. Furthermore any previous antibacterial treatment (both local and systemic) had to be discontinued at least 3 months prior to study entry.

Skin sampling

We first examined the skin lesions of all patients under normal lighting conditions, then in a dark room under Wood's light. For PMH patients a red fluorescent hair follicle in lesional skin was marked and for acne patients a red fluorescent acne lesion was marked. The skin was then disinfected with 70% alcohol with chlorohexidin 0.5% to eliminate superficial skin flora. To ensure that the red fluorescent part was in the biopsies we took the biopsies (2 mm) from the marked skin spots in a dark room under Wood's light. The biopsy specimens were cut transversally and each half was swapped on blood agar plates and immediately transported under anaerobic conditions to the Department of Medical Microbiology, University of Amsterdam (Amsterdam, the Netherlands) for further anaerobic processing and culturing.

Culture procedures

The inocula were spread on the agar plates and incubated under anaerobic conditions for 48 hours at 37 C. Colonies with a morphology compatible with *Propionibacterium*

were subcultured and subsequently identified with a Gram stain and the API 20A system for identification of anaerobic bacteria (Biomérieux). Per biopsy specimen one colony was subcultured again and stored at minus 70 C, and later regrown on blood agar plates to be sent to the VU University Medical Center, Amsterdam, the Netherlands for further molecular DNA processing.

Reference strains

Besides the clinical isolates, *P. acnes* (DSM 1897, ATCC 6919), *P. granulosum* (DSM 20700, ATCC 25564), *P. avidum* (DSM 4901, ATCC 25577) and *P. propionicus* (DSM 43307, ATCC 14157) were included in this study as reference strains. These strains were purchased from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Braunschweig, Germany and cultured under anaerobic conditions in accordance with the manufacturer's instruction.

DNA extraction

Prior to extraction of DNA, pure bacterial colonies were suspended in 2 ml TE-1 buffer (10 mmol/l Tris-HCL, 1 mmol/l EDTA, pH 8.0) and adjusted to match a turbidity of 1 McFarland. The suspension was then incubated with 10 mg/ml lysozyme at 37°C for at least 1 h. Thereafter DNA was isolated following the tissue protocol of the QIAmp DNA mini kit (Qiagen GmbH, Hilden, Germany). Finally, the DNA was eluted in 100 µl AE buffer of the extraction kit and stored at -20°C until needed.

Genotyping of bacterial strains

Amplified-fragment length polymorphism (AFLP) DNA fingerprinting was performed for further molecular characterization of the bacterial strains from 10 acne and 11 PMH patients.

The AFLP procedure was carried out as described earlier. Briefly, bacterial DNA was restricted with two enzymes with simultaneous ligation of adaptors to the restriction site. The reaction mixture consisted of 10 ng DNA, of 1x T4 DNA ligase buffer, 0.5 mol/l NaCl, 0.5 µg bovine serum albumin, 2pmol of the *Eco*RI adaptor (Isogen Bioscience BV, Maarssen, the Netherlands), 20 pmol of the *Mse*I adaptor (Isogen Bioscience), 80 U of T4 DNA ligase, 0.2 U of *Eco*RI, 1 U of *Mse*I. After incubation at 37°C for 3 h, the mixtures were diluted 1:20 in 0.1x TE buffer. All other enzymes were purchased from New England Biolabs (Beverly, MA, USA).

For amplification of the restriction fragments, 5 µl of the diluted mixture was added to 5 µl of PCR mixture, which consisted of 1x PCR buffer (Applied Biosystems, Foster City, CA, USA), 2 mmol/l dNTPs (Promega Benelux, Leiden, the Netherlands), 15 mmol/l $MgCl_2$

(Applies Biosystems), and 20 ng of *Eco*-A primer (5'-GACTGCGTACCAATTCAC-3') and 60 ng of *Mse*-C primer (5'-GATGAGTCCTGAGTAAC-3'). Eco-A was fluorescently labelled with carboxyfluorescein (Eurogentec, Maastricht, the Netherlands). Amplification was carried out in a GeneAmp PCR System 9700 (Applied Biosystems) under the following conditions: 2 min at 72°C, followed by 12 cycles of 30 s at 94°C, 30 s at 65°C and 1 min at 72°C and then 23 cycles of 30 s at 94°C, 30 s at 56°C and 1 min at 72°C, ended by a single extension at 72°C for 1 min.

Before analysis on an ABI Prism 3100 automatic sequencer, 2·5 µl of each PCR product was added to 22 µl Hi-Di formamide and 0·5 µl GeneScan-500 ROX standard (Applied Biosystems). Data were analyzed from 200-500 bp fragments with the Bionumerics software package, version 3·0 (Applied Maths, Sint-Martens-Latem, Belgium). Similarity coefficients were calculated with Pearson correlation and dendrograms were obtained by the unweighted pair group method using arithmetic averages (UPGMA) clustering.

Strain variation within patients

To exclude the presence of a mixture of different species in one patient, we resampled 3 acne patients and newly sampled 3 PMH patients, based on the same inclusion criteria as described above. This time a maximum of ten isolates per patient was submitted to DNA fingerprinting by AFLP and compared to the initial AFLP pattern, instead of only one isolate as in the prospective study.

16S rRNA gene sequencing and amplification

To confirm our AFLP findings of different (sub) species *16S rRNA* gene sequencing was performed on 2 isolates (from 1 acne and 1 PMH patient) from DNA group 1, 1 (from 1 acne patient) from group 2 and 3 (from 3 PMH patients) from group 3. For sequencing of the 16S DNA, a polymerase chain reaction was performed using the universal primers described by Mohammadi *et al*. (2005), which target a conserved region of 16S ribosomal DNA. A 20 µl PCR mixture consisting of 2·5 µl 10x PCR buffer, 0·5 µl 10 mmol/l dNTPs, 1·5 µl 25 mmol/l MgCl2, 25 pmol of each of the forward and reverse primer (Eurogentec), 0·2 µl Amplitaq gold (5 U/µl) and 5 µl template DNA was amplified under the following conditions: 10 min at 95°C followed by 30 cycles of 30 s at 95°C, 30 s at 60°C and 45 s at 72°C and a final step of 10 min at 72°C. PCR products were analyzed on 2% agarose gel in 1x Tris–Borate–EDTA (TBE) buffer (Life Technologies Ltd, Paisley, UK). After amplification the PCR products were purified using the QIAquick PCR purification kit (Qiagen).

The purified products were sequenced using primers covering a 466 bp fragment (F/R universal primer) and a 900 bp fragment (gd1 (5'-TGCTTTCGATACGGGTTGAC-3') and bak 4 (5'-AGGAGGTGATCCARCCGCA-3') (Dasen *et al*. 1998) from the 16S rDNA, resulting in a consensus sequence of about 1200 bp. Primers were obtained from Eurogentec.

Sequencing was performed using Big Dye terminator sequencing kit (Applied Biosystems). The programme consisted of: 10 s at 96°C, 5 s at 56°C and 4 min at 60°C for 25 cycles. The sequence products were purified and analyzed on an ABI 3100 automated DNA sequence analyser (Applied Biosystems). The *16S rRNA* sequences were aligned and compared with *P. acnes* ATCC 6919 (GenBank accession number AB042288) with the Bionumerics software package, version 3·0.

Biochemical analysis and antimicrobial resistance pattern

To determine whether our findings at the DNA level correlated with biochemical characteristics 6 isolates, 2 out of each DNA group were analyzed with the rapid ID 32A multitest identification system (Biomérieux, Lyon, France). In addition we assessed the antimicrobial sensitivity pattern of the strains by conventional methods.

RESULTS

Patients' characteristics are presented in Table I.

Culture and provisional conventional identification

Anaerobic culture on blood agar plates of biopsy specimens from both acne and PMH patients showed a remarkably homogenous growth of colonies compatible with *Propionibacteria*, with usually only very few other colony types. In all biopsy specimens the predominant colony type proved to consist of Gram positive rods that were identified as *P. acnes* by the API 20A system.

Genotyping bacterial strains

The AFLP patterns for *Propionibacterium* can be divided in three windows of similarity (Mohammadi *et al*. 2005). The first window, between 55% and 100%, defines strains of the same species; the second window, between 30% and 55%, defines strains of *related* species, possibly different subspecies; the third window, below 30% defines strains of *different* species (Savelkoul *et al*. 1999). Within the first window (55 to 100%) strains of the same species can be defined as identical (>90% similarity) or of different type. In our experiments strain characterization by

AFLP identified three groups (Figure 2): group 1 comprised strains isolated from 8 acne patients and 6 PMH patients. The isolates showed a similarity between 55 and 100% with the reference *P. acnes* strain. Group 2 comprised strains from 2 acne

Table I Characteristics of the study population

Characteristics	acne patients	PMH patients
No. patients	10	14
Female, No.	4	12
Male, No.	6	2
Mean age[1], Y	30 ± 5,3	27 ± 6,5
Skin phototype[2]		
III	6	9
IV	4	3
V	0	2

[1] Mean ± SD. [2] According to Fitzpatrick: determined by constitutive skin colour (the genetically determined colour or absence of colour in skin unexposed to solar irradiation and by facultative skin colour (skin colour that results from ultraviolet radiation exposure).

patients, showing a similarity level between 30 and 55% with the reference *P. acnes* strain. Isolates from group 3 comprised strains isolated solely from PMH patients (n = 8). For these strains a similarity level < 30% with the reference *P. acnes* strain was observed. All clinical strains showed a similarity level of < 30% when compared with *Propionibacterium avidum* (*P. avidum*), *Propionibacterium granulosum* (*P. granulosum*) and *Propionibacterium propionicus* (*P. propionicus*).

Strain variation within patients

We subjected 29 bacterial isolates cultured from the skin of 3 acne patients to the AFLP fingerprinting technique. All showed a similarity > 30% with the reference strain *P. acnes,* and fell into DNA group 1/2. Thirty colonies isolated from the skin of 3 PMH patients were also examined. All isolates (20) of 2 patients showed a similarity level < 30% with the *P. acnes* strain, comparable with bacteria in DNA group 3. The 10 bacterial isolates from the other PMH patient showed a similarity between > 55% with the reference strain *P. acnes,* comparable with bacteria in group 1 (Figure III).

16S rRNA gene amplification and sequencing

Comparison of the sequence of the *16SrRNA* gene of the various strains with the sequence of the *16SrRNA* gene of the *P. acnes* reference strain ATCC 6919 showed one nucleotide difference at position 827 with the isolates of group 2, and

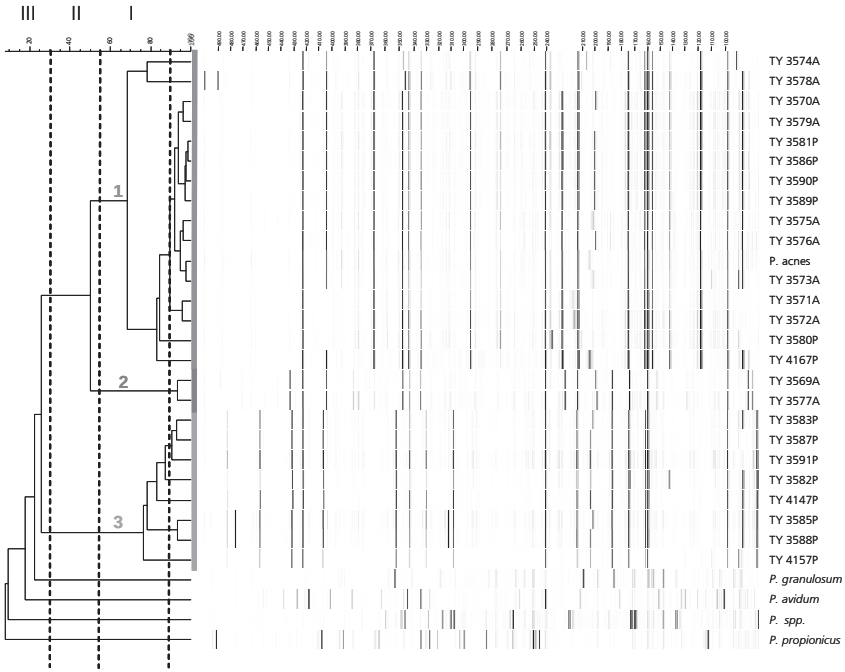


Figure 2 UPGM dendrogram derived from the AFLP patterns of 25 isolates of all 24 acne and PMH patients (from one acne patient two different bacterial isolates were obtained: TY 3571 / TY3572). Reference strains P. acnes, P. granulosum, P. avidum and P. propionicus were included in the analysis. Banding pattern of band sizes between 200 and 500 bp were analyzed. Three different DNA groups are indicated in the dendrogram by the numbers 1,2 and 3 and the coloured lines. In addition three windows of similarity were determined. Window I (8 acne patients and 6 PMH patients): homology between 55-100% suggests that the isolates are of the same species; Window II (2 acne patients): homology between 30-55% suggests that the isolates are of related species, possibly sub-species; Window III (8 PMH patients): <30% homology indicates isolates of a different species, forming a clear distinct DNA group.

1 nucleotide difference at position 1243 with the isolates of group 3. One isolate (TY 3585) of DNA group 3 had an additional nucleotide difference at position 712 besides the one at position 1243 (Table II).

Biochemical analysis and antibiotic resistance pattern

Two isolates from each DNA group were analyzed with the rapid ID 32A system. Isolates from group 1 and 2 were identified as *P. acnes* with a certainty of 99.9%. The isolates from group 3 were not identified as *P. acnes* by the computer, but were labelled with an "un-acceptable profile" since both isolates showed negative results

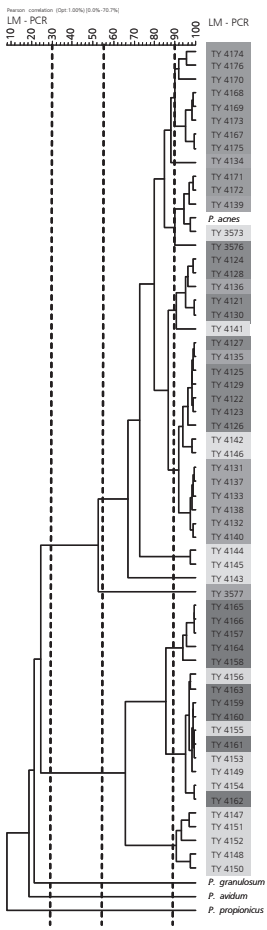


Figure 3 AFLP pattern of various strains isolated from the same patient (intrapatient strain variation): UPGM dendrogram derived from 59 isolates of 3 acne patients and 3 PMH patients. The colours represent the strains per patient. Yellow, Pink and green represent the acne patients; Blue, purple and orange represent the PMH patients.

Table II Nucleotide differences of bacterial isolates compared to the reference strain[1]

Nucleotide	Ref strain	TY 3574	TY 3589	TY 3569	TY 3582	TY 3585	TY 3591
Position	ATCC 6919	group 1	group 1	group 2	group 3	group 3	group 3
712	G	*	*	*	*	A	*
827	T	*	*	C	*	*	*
1243	G	*	*	*	A	A	A

[1] 16S rRNA gene sequence analysis results of bacterial isolates from 1 acne and 1 PMH patient (group 1), 1 acne patient (group 2) and 3 PMH patients (group 3). * = reference nucleotide position; the ATCC strain belongs to group I. A = Adenine, G = guanine, C = cytosine, T =thymine.

for Pro A, a substance that always shows positive results for *P. acnes*. Sensitivity and resistance analysis of all strains were similar and typical for P. *acnes*.

DISCUSSION

In the present study we showed by AFLP DNA typing that bacteria cultured from 8 out of 14 PMH patients were substantially different from the *P. acnes* bacteria seen in acne. We classified these bacteria as DNA group 3. By conventional culturing techniques, both in this study and in a previous study (Westerhof *et al*. 2004), the microbial species (*P. acnes*) found in PMH could not be differentiated from that found in acne. Acne, however, is rarely seen in patients with PMH and vice versa. Interestingly, these group 3 bacteria were not detected in any of the acne patients. Furthermore, *16S rRNA* gene sequencing and biochemical tests confirmed that there are differences between the groups of bacteria, although the differences are minor. We therefore believe that there is a relationship between PMH and the *Propionibacteria* clustering in group 3.

In 2005 Mohammadi *et al*. also described three different types of *P. acnes* strains. They conducted a study to determine the source of bacterial contamination of platelet concentrates (PCs). *P. acnes* isolates derived from PCs and corresponding red blood cells concentrates (RBCs) were analyzed by AFLP procedures and by *16S rRNA* gene sequencing. These authors describe three bacterial groups that are comparable to the bacterial groups we found in our study: group I and II were *P. acnes* (sub) species (30-90% homology with the reference *P. acnes* strain) and group III showed < 30% homology with the reference strain *P. acnes*, suggesting a different species. Furthermore, 16S r RNA gene sequencing showed similar differences as those we detected: at position 827 group I bacteria showed a T nucleotide while group II bacteria showed a C nucleotide. These findings also correspond to the findings of McDowell *et al*. 2005 who observed the same difference in nucleotides at position 827. *16S rRNA* gene sequencing results of the group 3 bacteria in our study corresponded with the group III bacteria described by Mohammadi *et al*. 2005. In their study, at position 1243 group III bacteria showed an A nucleotide, while the reference *P. acnes* strain showed a G nucleotide.

Analysis by AFLP showed that the *P. acnes* bacteria (group 1) were quite different from the bacteria found solely in PMH patients (group 3). The level of identity was that of a different *Propionibacterium* species. This observation was confirmed by the differences in *16S rRNA* gene sequencing and biochemical tests; the differences

were minimal however, in combination with the similar antimicrobial sensitivity pattern of the two bacterial groups, this suggests that we might be dealing with a bacterium of the genus *Propionibacterium* but of a yet undefined species.

McDowell *et al*. 2008 recently also described a new phylogenetic group of *P. acnes* which they called *P. acnes* type III. This strain had a 99.8 to 99.9% identity to type I and type II *P. acnes* strains (Relyveld *et al*. 2006), when analyzed by *16S rRNA* gene sequencing. However immunofluorescence microscopy, sequencing of the *16S rRNA* gene and the specific recA gene (a protein-encoding gene with housekeeping functions), and biochemical analysis of the type III *P. acnes* bacteria showed obvious differences between these bacteria and the type I and II bacteria. When we compare their *16S rRNA* sequences to our results, the same point mutation at nucleotide position 1243 is observed, indicating that the *P. acnes* type III corresponds to our DNA group 3.

It has to be noted that in 6 PMH patients we found group 1 *P. acnes* bacteria and not group 3 bacteria. However, this does not exclude group 3 bacteria from being present on the skin of these patients. Possibly they were present in low numbers, or sampling error may have occurred.

Group 3 bacteria were isolated solely in PMH patients, and not in acne patients. This suggests a relationship between PMH and these bacteria. The findings may help explain why not all acne patients have PMH and vice versa, and why PMH can best be treated with antimicrobial therapy. Furthermore this study shows that conventional identification methods are not sufficient to distinguish some species or subspecies of specific genera that may play an important role in the pathogenesis of diseases. Further research is necessary to substantiate the importance of the role of this putative new *Propionibacterium* species in PMH.

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4 CLINICAL CHARACTERISTICS IN PATIENTS WITH PROGRESSIVE MACULAR HYPOMELANOSIS

ABSTRACT

Introduction: To gain a more accurate description of progressive macular hypomelanosis in order to improve the recognition of the disease and to diminish over and under-treatment.

Patients and Methods: Design: Survey. Setting: The Netherlands Institute for Pigment Disorders. Patients: 152 patients diagnosed with progressive macular hypomelanosis. Main Outcome Measure: Results based on 101 questionnaires returned.

Results: 74% returned a completed questionnaire (101 of 137 eligible participants). In 76% of the patients PMH was not diagnosed at the first doctors' visit for the complaints. In 98% of all patients the disease was symmetrically spreading on the trunk and in a few to the face and proximal extremities. Most patients with PMH had a dark skin type (III to VI). In the majority of patients PMH was initially diagnosed and treated as pityriasis versicolor.

Conclusion: Our study gives insight in the clinical course of PMH and we provide a complete description of the clinical features and a guideline for physicians. Hopefully this will improve the accuracy of the diagnosis in patients with hypopigmented macules.

INTRODUCTION

Progressive macular hypomelanosis (PMH) is known as a distinct entity for 20 years but still many physicians are unfamiliar with this disease. Therefore PMH is often misdiagnosed and consequently often treated inadequately. Incidence and prevalence of PMH are unknown. In our tertiary referral center the Netherlands Institute for Pigment Disorders (SNIP) each year in approximately 2% of all patients PMH is diagnosed.

PMH is characterized by ill-defined nummular hypopigmented spots, symmetrically localized on the front and back of the trunk, sometimes extending to the extremities, the neck, and the face (Figure 1) (Relyveld *et al*. 2007). Usually, patients complain about cosmetic inconvenience, in some cases even leading to social restrictions as a consequence of feelings of shame.

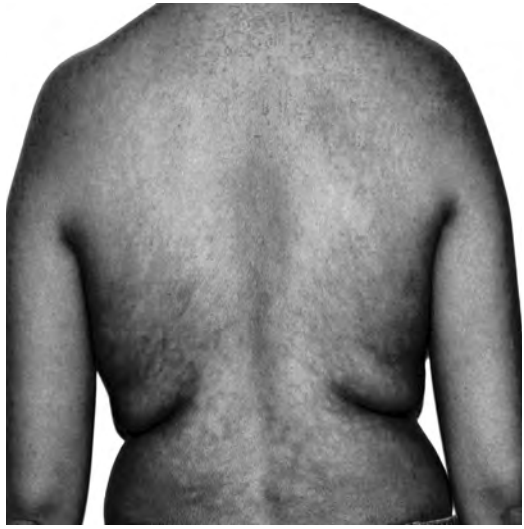

Figure 1 28 year old female with PMH.

In 2004 we provided an additional diagnostic criterion to the clinical characteristics for PMH mentioned above. We discovered that when the lesional skin was illuminated with Wood's light in a dark room, small spots of red follicular fluorescence could be observed. This fluorescence was absent in adjacent normal skin (Westerhof *et al*. 2004). Red follicular fluo-rescence is characteristic for *Propionibacterium acnes* bacteria. These bacteria are considered to be commensal flora and are present in sebaceous rich areas of the skin. When these areas are illuminated with Wood's light in a dark room, red follicular fluorescence can be observed. It was hypothesized that these bacteria might be responsible for the hypopigmentations in PMH. This hypothesis was supported by a prospective study in which *Propionibacterium acnes* could be cultivated from the lesional skin in patients with hypopigmented spots, while specimens taken from the normal skin did not show growth (Westerhof *et al*. 2004).

In previous studies we showed the differences in the histology, electron microscopy and treatment between PMH and other skin disorders resembling PMH, such as pityriasis versicolor and pityriasis alba (Relyveld *et al*. 2006, 2007, 2008). Despite several descriptions of the characteristics of PMH that can be found in the literature (Table I) (Guillet *et al*. 1988, Borelli 1987, Lesueur *et al*. 1994, Menke *et al*. 1997, Kumarasinghe *et al*. 2006) there is no consensus with regard to the description of the disease. Therefore we developed a questionnaire for a survey among patients in whom PMH had been diagnosed. The results of this questionnaire, together with the findings of the clinical descriptions of PMH from the literature will be used to provide a more thorough description of the natural course and clinical characteristics of PMH. The results will help physicians to distinguish PMH from diseases with similar symptoms, such as pityriasis alba and pityriasis versicolor. This will hopefully result in an increase in the number of accurate diagnoses and consequently reduce the amount of over- and under-treatment.

METHODS

Questionnaires

The questionnaire contained questions concerning the patients' demographic characteristics and their skin type, questions concerning the onset of the disease, family history, questions concerning the clinical features of the disease, the progression of the disease (for this question the patients were asked to depict the sites of the lesion and sites of the progression on a full body figure), accompanying symptoms and former treatments. Furthermore we asked about patients' medical history to detect the existence of diseases possibly related or similar to PMH.

Inclusion criteria

All patients diagnosed with PMH between January 1998 and April 2004 by dermatologists at the SNIP received a questionnaire. The diagnosis of PMH was based on the clinical description of non scaly, hypopigmented macules, symmetrically distributed on the trunk and/or face and a coexistence of red follicular fluorescence present in hypopigmentations and absent in adjacent normal skin.

Human subjects committee approval was not required for this study and was therefore waived.

Table I Several descriptions of PMH characteristics in the literature

Author	Clinical description	Localization	Patients	Additional
Guillet *et al.* (1985, 1988, 1992)	- Hypopigmented macules	- Back, sometimes abdomen, absent on dorso-lumbar line	- Mainly women - Mixed (casoid-negroid) ancestry - Age 18-25 years	- No response to various topical treatments, including anti-septic agents, anti-fungal preparations and corticosteroids
Borelli (1987)	- Hypopigmented macules irregular and round with a diameter of 0.6 to 2 cm, symmetrically distributed	- Trunk, especially in the lumbar, sacral and epigastric regions, sometimes on the flanks	- Both sexes - Brown-skinned - Age 14-28 years	- No other symptoms
Lesueur *et al.* (1994)	- Hypopigmented macules of 0.5-3.0 cm	- Trunk	- Mainly women - Adults of ethnic origin Higher prevalence in people with lighter skin - Age 17-48 years	- Spontaneous regression after an average of 25 years - Repigmentation only after sun exposure - Usually the hypopigmentations reappeared
Menke *et al.* (1997)	- Ill-defined nummular hypopigmented spots with a diameter of 0.5-2 cm	- Trunk, symmetrically localized with often a confluent hypopigmented area on the front and backside - No scaling		- No spontaneous regression - Lesions still present after 10 years - Stable or slow progression over the years
Kumarasinghe *et al.* (2006)	- Hypopigmented, ill-defined, discrete or confluent macules	- Lumbar region, abdominal wall, lower flanks. Occasionally on buttocks, arms and upper chest	- No predominant sex distribution - Chinese, Mongoloid and Indian type (patients from India, Bangladesh and Sri Lanka) - Adolescents and young adults - Virtual absence in the elderly	- Exposure to sunlight enhanced visual demarcations between normal and lesional skin

Analysis

Survey responses were entered into a database by one independent investigator. If this person was not sure about an answer, a second investigator was asked. Discrepancies were resolved by consensus.

Literature search

The following search engines were used for the literature search on PMH: pubmed, up to date, google and web of science. All publications, regardless of the methodology, were used.

RESULTS

Patients' reply, characteristics and demographics

Between April and June 2004, questionnaires were sent to 152 patients with the diagnosis PMH. 15 (10%) were undeliverable because the mailing addresses were not correct. 36 (26%) were not returned, therefore 101 (74%) of the 137 patients who received a questionnaire did send back a completed questionnaire. Patients' characteristics and demographics are presented in Table II.

Table II Patients' characteristics and demographics in 101 patients with PMH who returned a questionnaire

Characteristics	N = 101
Female, No. (%)	83 (82)
Median age (IQR)	27 (23-33)
Median age of onset (IQR)	19 (15-23)
Skin type*, No. (%)	
II	3 (3)
III	20 (20)
IV	23 (23)
V	36 (36)
VI	18 (18)
Median duration of PMH in years (IQR)	7 (4-11)

IQR = inter quartile range, * skin type according to Fitzpatrick.

Diagnosis at first doctor's visit

Seventy seven (76%) patients answered that PMH was not diagnosed at once. Of the 24 (24%) patients who were diagnosed with PMH at once, 13 (54%) were

diagnosed by a dermatologist at the SNIP, 9 (38%) patients were diagnosed by other dermatologists and in only 2 (8%) were diagnosed by a general practitioner. Of those patients in whom PMH was not diagnosed at the first visit a yeast infection was diagnosed in 40 (52%) patients. In 6 (8%) no diagnosis was made. The other diagnoses were vitiligo in 4 (5%) and eczema in 3 (4%) patients; bacterial infection, pigmentary disorder, hypopigmentation as a result of vitamin D deficiency, and hypopigmentation due to the use of oral contra-conceptives were all mentioned by only 1 (1%) patient.

Clinical characteristics and course of PMH

In 88 (87%) of all patients the disease started on the trunk and progressed by spreading symmetrically further over the trunk and sometimes to the face and proximal extremities (Table III).

Sixty eight (67%) patients observed confluence of hypopigmented macules. In all these 68 patients, confluence appeared on the trunk and in 7 (10%) of those patients confluence also appeared in the face. In 59 (58%) of all patients (treated

Table III Locations and progression of PMH lesions in 101 patients with PMH who returned a questionnaire

Affected body sites	First appearance (no. of patients)	Areas of spreading of macules (no. of patients)
Trunk	88	45
Face	10	1
Proximal arms	3	1
Trunk/Face	0	5
Trunk/Proximal arms	0	25
Trunk/Proximal legs	0	5
Trunk/Proximal arms/Proximal legs	0	11
Trunk/Face/Proximal arms	0	2
Trunk/Face/Proximal legs	0	1
Trunk/Face/Proximal arms/Proximal legs	0	2
Proximal arms/Proximal legs	0	1

"First appearance" represents area(s) on the body where hypopigmented macules were first seen. "Spread" represents area(s) on the body to where macules later further spread to. The area of the neck is included in the area of the face.

and not treated for the disease) PMH did not disappear. Nine (9%) patients were not sure whether the lesions were gone or not.
The lesions resolved in 32 (32%) of all PMH patients due to treatment. According to 11 (34%) of those patients the lesions disappeared as a result of treatment with a combination of benzoyl peroxide, fluticasone and UVA phototherapy.
In 14 (44%) of the 32 patients in whom PMH resolved, the lesions returned after a certain period of time.
Forty nine (49%) patients answered that sunlight made the macules more obvious, while 31 (31%) patients answered less obvious. Thirteen (13%) of all patients had accompanying symptoms. Of those 13 patients, 1 (1%) patient mentioned rash (not further defined), 4 (4%) patients mentioned itching, 1 (1%) patient mentioned dry, scaly, painful skin and 3 (3%) patients mentioned dry, scaly, itching skin, 2 (2%) patients mentioned dry skin, 1 (1%) patient mentioned dry, scaly skin and 1 (1%) patient mentioned psychological problems that were not further defined.

Co-morbidity

Patients reported to have had asthma (7), hay fever (38), psoriasis (3), atopic dermatitis (11), fungus or yeast infection of the skin (18) and leprosy (1).
Other diseases that were mentioned were: bronchitis 1 (1%) patient, hypoglycemia 1 (1%) patient, stomach pain 1 (1%) patient, melasma 3 (3%) patients, naevus depigmentosus 1 (1%) patient, post inflammatory hypopigmentation after eczema 1 (1%) patient, allergies 1 (1%) patient, verrucae seborrhoicae 1 (1%) patient and vitiligo 1 (1%) patient.

Family history

Twenty three (23%) patients reported to have family members with PMH. Nineteen (83%) of which were brothers and sisters. Forty seven (47%) patients did not have family members with PMH and 31 (31%) patients did not know if any other family members had PMH.

DISCUSSION

With the results of this study we are able to give a more accurate and more complete description of PMH. This will improve the diagnostic accuracy of the disease.

Comparison of our results with the description of others

We found that PMH is mainly diagnosed in adolescent and adult women with a skin type III to VI, which is similar to findings in other studies (Relyveld *et al*. 2007,

Borelli 1987, Lesueur *et al*. 1994). Other studies (Borelli 1987, Lesueur *et al*. 1994, Menke *et al*. 1997, Kumarasinghe 2006) found that the hypopigmented macules where mainly localized on the trunk. Patients in our study also reported macules in the face and the proximal extremities. In agreement with other studies (Borelli 1987) is however, that besides the hypopigmented macules, only a few patients reported accompanying symptoms. Like other studies (Lesueur *et al*. 1994, Menke *et al*. 1997, Kumarasinghe *et al*. 2006) we also found that spontaneous remission seems not to occur within five years after the onset of the disease.

Limitations of the study

Some aspects of our study require comment. Firstly there are no official diagnostic criteria for PMH available yet. The diagnosis was therefore based on our own criteria, which showed in former studies to be of significant value in excluding other disorders like pityriasis alba and pityriasis versicolor (Westerhof *et al*. 2004, Relyveld *et al*. 2006, 2008).

Furthermore our patients were recruited in a tertiary referral center. It is not sure that this population resembles the general population with PMH. For example the female to male ratio in our population is 8:1, which might be different from the ratio that can be found in the general population. It is likely that relatively more men are affected by PMH than our data show. The fact that PMH is first of all perceived as a cosmetic disorder, might explain why mostly women are seen at our institute. However, Lesueur *et al*. (1994) performed a screening of 511 patients for leprosy, of whom 121 eventually had PMH and those patients were mainly women. This group was not a pre-selected PMH group, suggesting that PMH may indeed be a disorder that affects mainly women. Finally, hormonal status in women can result in an increase in sebum production, making female skin a favorable environment for *Propionibacteria* to grow in.

We also have to consider the possibility of selection bias though, since we do not know if there are patients who might have been successfully treated by their general practitioner and therefore have not been referred to the SNIP or patients who did not consider the macules as a problem and did not visit a physician at all.

Recommendation and conclusion

Clinical picture and clinical course of PMH, based on this survey

PMH is mainly seen in women in their adolescent or adult years. This observation can be substantiated by the fact that we already showed in a previous study (Westerhof *et al*. 2004) that *Propionibacterium acnes* might be the causative organism of PMH.

Table IV Clinical and diagnostic characteristics of PMH based on previous studies and a survey among 101 patients with PMH

Consider PMH when a patient presents with the following characteristics:
Adolescents, young adults
Symmetrically distributed, hypopigmented, non scaly macules mainly on trunk sometimes face and proximal extremities, often confluence on the trunk
Red follicular fluorescence in lesional skin. No fluorescence in adjacent normal skin2
Gram positive rod-like bacteria in sebaceous ducts and glands of lesional skin, but absent in normal (peri-lesional) skin (Westerhof *et al.* 2004)
Hardly ever spontaneous repigmentation; if so then usual reappearance in same areas

These bacteria are residents of the pilosebaceous ducts and prefer a sebum-rich environment. Sebum production is highest in adolescent years and decreases around late adult years. On the other hand adolescents and young adults might be more concerned about their appearance than older people.

The disorder is mainly seen in patients with darker skin types (III - VI); however patients with lighter skin types may also be affected. An explanation for this difference may be that in lighter skin types the hypopigmented macules are much less obvious and therefore patients do not even notice the lesions or do not find it worth visiting the doctor.

PMH originates on the trunk, spreads further centrifugal on the trunk and sometimes to the face and the proximal extremities. The distribution of the lesions is also in concordance with the distribution of sebum-rich areas and therefore the presence of *Propionibacteria* in the skin.

Usually it does not resolve and if it does, it reappears mostly in the same areas of the skin. In Table IV the clinical and diagnostic characteristics of PMH are summarized.

Only 1 to 4% of the patients mentioned subjective symptoms such as pain and pruritus. And only 7% mentioned objective symptoms such as rash or scales. It is unknown how many people have these symptoms in the general population, but there seems to be no significant correlation between PMH and these symptoms.

The prevalence of other skin diseases is not higher in patients with PMH compared to the general population according to figures of a Dutch Health Surveillance Institute (RIVM) with respect to the prevalence of eczema, asthma, allergic rhinitis and psoriasis (RIVM 2008). Therefore it is not likely that there is a relationship between PMH and other skin diseases.

Unfortunately there is a lack of information about the prevalence of yeast infections in the Netherlands. Since only one fifth of the PMH patients mentioned a previous yeast infection, we conclude that there is no correlation between such an infection and PMH.

This study shows that PMH is unfamiliar to many physicians and it is mostly diagnosed and treated as a yeast infection and in some cases as eczema or vitiligo. Since vitiligo is characterized by depigmented macules, which are easy to differentiate from the hypopigmented macules in PMH, PMH seems most difficult to distinguish from pityriasis versicolor and pityriasis alba.

Table V Most important differences between PMH, PV and PA based on previous studies and a survey among 101 patients with PMH

Disorder	Patient characteristics	Diagnostic features	Clinical course
PMH	Adolescents and young adults Possibly a genetic component	Symmetrical, hypopigmented macules, mainly on the trunk, No other objective or subjective symptoms Red follicular fluorescence in hypopigmented macules under Wood's light Gram positive rod-like bacteria in sebaceous ducts and glands of lesional skin, but absent in normal (peri-lesional) skin3	Progression on the trunk, sometimes to the face and proximal extremities No effect of anti-mycotic or anti-inflammatory treatment If lesions resolve, recurrence in same areas
PV	Adolescents and young adults No genetic component	Asymmetrical hypopigmented, scaly macules on the trunk, sometimes elsewhere Yellow fluorescence of macules under Wood's light KOH preparation: positive (spores and mycelia)	Can be progressive further on trunk Good effect of antimycotic treatment Recurrence usually on different parts of the trunk and elsewhere
PA	Children Probably a genetical component (often seen in atopic patients)	Starts with erythematous asymmetrical macules, evolves in hypopigmented, scaly macules on the trunk, proximal arms and face No fluorescence under Wood's light	Usually resolving in adolescent years Good effect of anti-inflammatory treatment Recurrence usually on different parts of the trunk, face, proximal arms

PMH = progressive macular hypomelanosis, PV = pityriasis versicolor, PA = pityriasis alba.

In other studies we showed that there are differences in histological, ultrastructural and treatment characteristics (Relyveld *et al*. 2006, 2008) between PMH on the one hand and pityriasis versicolor and pityriasis alba on the other hand. The underlying study shows additional differences in the clinical characteristics and course of these three disorders (Table V).

A genetic factor can not be ruled out in the pathogenesis of PMH, since a quarter of all patients mentioned family members with PMH and a third did not know if they had family members with PMH.

It is inevitable that further research into the pathogenesis, diagnosis and treatment of PMH is essential.

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Ultrastructural findings in progressive macular hypomelanosis indicate decreased melanin production. *J Eur Acad Dermatol Venereol*. 2008;22(5):568-574

5 ULTRASTRUCTURAL FINDINGS IN PROGRESSIVE MACULAR HYPOMELANOSIS INDICATE DECREASED MELANIN PRODUCTION

ABSTRACT

Introduction: The pathogenesis of progressive macular hypomelanosis (PMH) is unknown. Recently, Westerhof *et al*. (2004) hypothesized that Propionibacterium acnes produces a depigmenting factor that interferes with melanogenesis in the skin, resulting in hypopigmented spots. The purpose of the study is to gain an insight into the pathogenesis of PMH.

Patients and Methods: We took a biopsy of 2-mm diameter from normal and lesional skin in eight PMH patients. Using electron microscopy, we compared melanization of melanosomes, melanosome transfer and amount of epidermal melanin in normal and lesional skin.

Results: Compared to non-lesional skin, we observed a decrease of epidermal melanin and less melanized melanosomes in lesional skin of all patients. When comparing normal and lesional skin of patients with skin type V and VI, we observed a difference in melanosome size and maturation and a switch of transferred melanosomes from single stage IV transferred melanosomes to aggregated stage I, II and III transferred melanosomes, as seen in healthy skin of skin type I to IV.

Conclusion: Hypopigmentation in PMH seems to be the result of an altered melanogenesis based on a decrease in melanin formation and a change in the distribution of melanosomes. In lesional skin of PMH patients with skin type V and VI less melanized, aggregated melanosomes in stead of single, mature melanosomes are transferred from melanocytes to keratinocytes. This results in a decrease of epidermal melanin. Further investigations are needed to determine the precise role of Propionibacterium acnes in this alteration of melanogenesis.

INTRODUCTION

Progressive macular hypomelanosis (PMH) is a skin disorder occurring in young adults. It is characterized by symmetrically distributed ill-defined nummular hypopigmented macules mainly on the trunk, sometimes extending to the neck and face, the buttocks and the upper half of the extremities (Figure1). PMH is often misdiagnosed as pityriasis versicolor (PV) and pityriasis alba (PA).

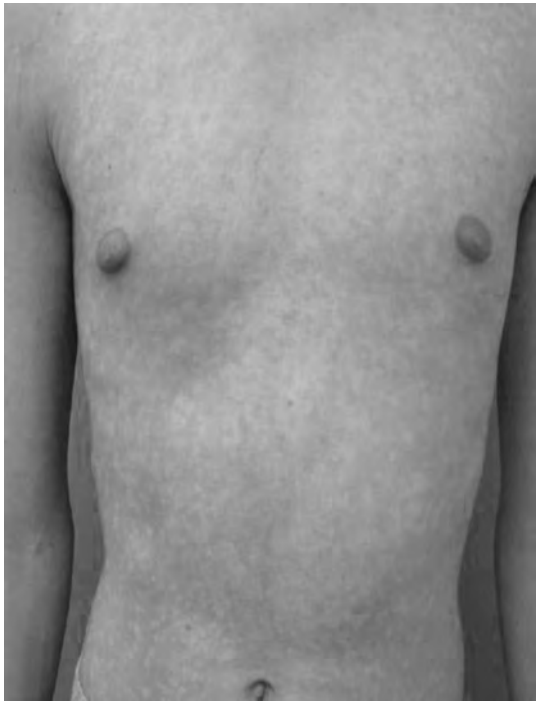

Figure 1 A 22-year-old male patient with PMH (skin type V).

The pathogenesis of PMH is unknown. Guillet *et al*. (1988) conducted ultrastructural studies of lesional and non-lesional skin in two PMH patients. They showed the presence of stage IV single melanosomes (the types of melanosomes normally present in black skin) in the healthy looking skin, and stage I–III aggregated melanosomes (the types of melanosomes normally present in white skin) in the hypopigmented spots. Because they observed PMH in racially mixed (Negroid-Caucasoid) patients, they concluded that it is caused by a 'melting' of genes of white and black parents. In 2006, Kumarasinghe *et al*. (2006) published an article which describes the clinico-pathological findings in PMH patients.

Recently, Westerhof *et al*. (2004) proposed that Propionibacterium acnes, by producing a depigmenting factor, might be the causative organism. This view is based on the fact that during inspection of the skin of PMH patients in a dark room with Wood's lamp, a red follicular fluorescence was observed restricted to the lesional skin. P. acnes was cultured from biopsies taken from follicular lesional skin in 7 of 8 patients, whereas from healthy skin, no bacteria were cultured. This

hypothesis is supported by our finding that treatment of PMH patients with topical antibacterial agents leads to a significantly better improvement than treatment with anti-inflammatory agents (Relyveld *et al*. 2006).

From a theoretical point of view, a variety of genetic and environmental factors can be envisaged to lead to a decrease in epidermal melanin:

(1) Disorders in the melanization of melanosomes due to a defect in the structure of tyrosinase or an inhibition of tyrosinase: a decrease in the melanin synthesis leads to disorders in maturation, size and distribution of melanosomes, followed by hypopigmentation.

(2) Disorders of maturation of melanosomes: the formation of melanin is related to the structural maturing from premelanosomes to melanosomes. A decreased maturation of melanosomes leads to hypopigmentation.

(3) Disorders in melanosome transfer: microfibrils inside melanocyte dendrites and certain receptors are crucial for the transfer of melanosomes from melanocytes to keratinocytes. A decrease in the velocity of melanosome transfer from the melanocyte to the keratinocyte leads to hypopigmentation. Melanosome transfer may be disturbed by increased keratinocyte turnover and by all processes disturbing the interactions/contact between melanocytes and keratinocytes.

(4) Disorders in degradation of melanosomes: increased rate of melanosome destruction might lead to hypopigmentation

The purpose of our study is to gain an insight into the mechanisms involved in the pigmentation disturbance of PMH. We conducted an electron microscopic study to find out which of the four mechanisms underlies the hypopigmentation in lesional skin of PMH by comparing lesional and non-lesional skin of these patients.

PATIENTS AND METHODS

Patients and settings

Patients with PMH were seen at the Dermatology Department of the Saint Franciscus Hospital in Rotterdam, the Netherlands and at the Institute for Pigment Disorders in Amsterdam, the Netherlands. The diagnosis of PMH was based on a combination of the clinical signs, the presence of red fluorescence coinciding with the pilosebaceous ducts of lesional skin when examined with a Wood's lamp in a dark room, negative KOH tests and the absence of spores and mycelia on histological examination.

Diagnostic biopsies

Two biopsies of 2 mm for routine examination and electron microscopy were taken under local anaesthesia with xylocaine 2%, one from the affected skin and the other from the adjacent normal-appearing skin from the back of the patients.

Light microscopy

A part of each biopsy was fixed in 10% formaldehyde solution and embedded in paraffin. Paraffin sections were histologically stained with haematoxylin and eosin, and the difference in the amount of melanin in the lesional and normal skin was assessed.

Electron microscopy

For electron microscopy, part of the biopsies was fixed in Karnovsky's fixative, post-fixed in 1% osmium tetroxide (OsO_4) and further processed according to standard procedures.

Assessment of the number of melanosomes, the size of the melanosomes and melanosome transfer was done by comparing electron microscopic pictures of lesional and non-lesional skin. In order to confirm our clinical findings and more important, to improve our understanding of the pigment dynamics in PMH, the difference in total epidermal melanin between normal and lesional skin was estimated in one visual field according to the following assessment scale: > 50%, more than 50% less melanin in lesional skin than in non-lesional skin; 0% to 50%, between 0% and 50% less melanin in lesional skin than in non-lesional skin; 0, lesional and non-lesional skin have same amount of melanin. At a magnification of 4500×, three keratinocytes from lesional and from normal skin were selected. Each keratinocyte was then investigated at a magnification of ×44 000. Melanosome transfer type (single or aggregated) was determined and melonosome size was measured.

Former studies indicate a correlation between melanosome size and the distribution pattern of melanosomes within secondary lysosomes of keratinocytes. Melanosomes larger than 1 μm are singly distributed, whereas those smaller than 1 μm in long axis are aggregated to form melanosome complexes (Szabo 1969, Toda *et al*. 1972, Wolff *et al*. 1974, Yamamoto *et al*. 1994, Jimbow *et al*. 1998).

Based on these findings, we assessed the length of the long axis of the 10 largest melanosomes in each keratinocyte. Per patient the median of the length of the melanosomes in lesional and normal skin was then computed. The same calculations were also done for the diameter of the melanosomes.

The median and 25th and 75th percentile of the length and diameter of the melanosomes were described, but additional statistical tests are meaningless for a sample size of eight patients. Researchers performing the calculations were all blinded.

RESULTS

Subjects

Eight patients with PMH between 15 and 41 years (mean age, 26 years) were included. All except one were female. Baseline characteristics of the patients are presented in Table I.

Table I Baseline characteristics of patients

Female, no.	7
Male, no.	1
Mean age in years +/- SD	26.1 ± 8.2
Skin type *, no.	
III	2
IV	1
V	3
VI	2

*Skin type according to Fitzpatrick.

Light microscopy

A comparison of the pigmentation in normal and lesional skin revealed a decrease in the amount of melanin in the epidermis of the lesional skin of all patients. The dermis showed no abnormalities (Figure 2A,B).

Electron microscopy

General epidermal changes

In all patients, electron microscopic pictures of low magnification, and light microscopy showed a decrease in pigmentation of the epidermis in the lesional skin compared with the normal skin.

Changes in melanocytes

In both the lesional and the non-lesional skin of PMH patients, the melanocyte cell bodies contained melanosome precursors in all stages of development. In lesional

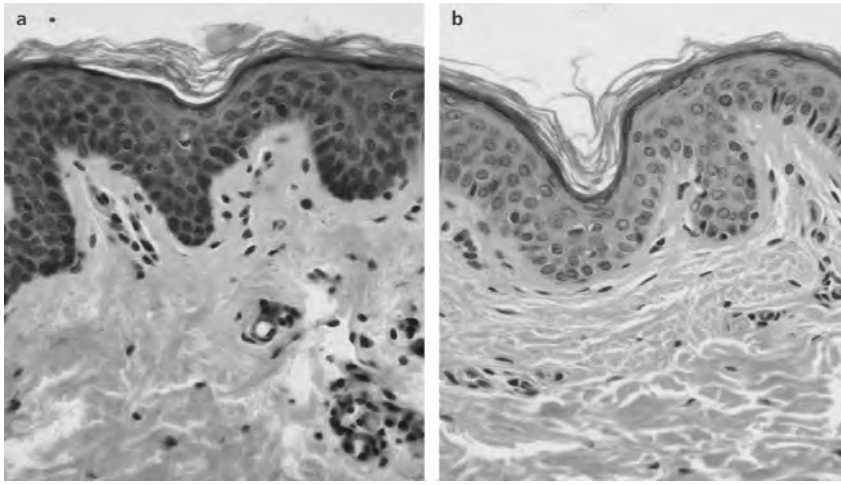

Figure 2 (A) Light microscopy of normal skin in a PMH patient with skin type V. (B) Light microscopy of lesional skin in the same patient.

skin; however, mature melanosomes were distinctly smaller and less melanized than those in non-lesional-skin. The melanocytic dendrites in the lesional skin of patients with skin types V and VI contained smaller, less melanized melanosomes than those in non-lesional skin. In patients with skin types III and IV, on the other hand, the melanocytic dendrites in normal as well as lesional skin showed similar melanosomes.

Changes in keratinocytes

Non-lesional skin Keratinocytes in patients with skin type V and VI showed numerous single, large, melanosomes that were ellipsoidal and intensely melanotic (Figure 3A). Patients with skin type III and IV showed single large stage IV melanosomes as well as clustered, immature stage II and III, aggregated melanosomes.

Lesional skin Keratinocytes in patients with skin type V and VI showed smaller and less dense melanosomes (stage II and III melanosomes) that were clustered in membrane bound groups (Figure 3B).

The median length as well as the median diameter of the melanosomes was longer in normal skin than in lesional skin of all skin types, except for the median length in skin type IV. Differences in melanosome size are presented in Table II.

The keratinocytes in patients with skin type III and IV had the same aspect as described above, although the differences between normal and lesional skin were

Table II Electron microscopic observations in keratinocytes in PMH patients

Patient number	Skin type	Amount of melanin LS vs non-LS*	Distribution non-LS**
1	III	>50%	Single, large, intensely melanotic, and few smaller, less melanotic in membrane bound groups
2	III	0-50%	Single, large, intensely melanotic as well as small, less melanotic in membrane bound groups
3	IV	0	Single, large, intensily melanotic
4	V	>50%	Single, large, intensely melanotic
5	V	0-50%	Single, large, intensely melanotic
6	V	0-50%	Single, large, intensely melanotic
7	VI	>50%	Single, large, intensely melanotic
8	VI	>50%	Single, large, intensely melanotic

LS: lesional skin; non-LS: non-lesional skin. *Amount of melanin in keratinocyte of LS compared with non-LS. 50%, more than 50% less melanin than in non-lesional skin; 0% to 50%, between 0% and 50% less melanin than in non-lesional skin; 0, lesional and non-lesional skin have same amount of melanin. (Percentages are based on estimations). **Distribution of melanosomes in keratinocytes of non-LS. ***Distribution of melanosomes in keratinocytes of LS. (P25–P75): 25th to 75th percentile.

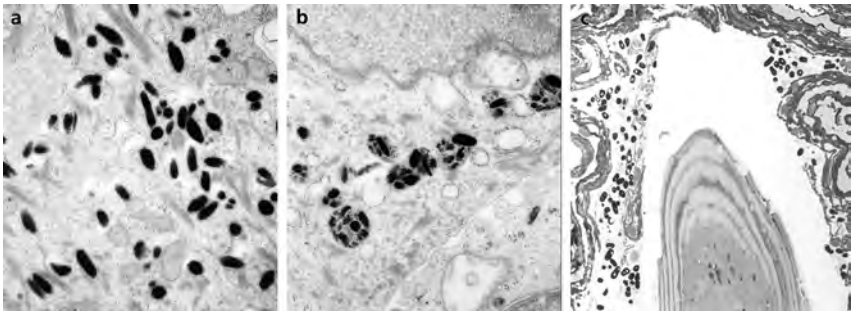

Figure 3 (A) Magnification ×44 000. A keratinocyte in non-lesional skin of a patient with skin type V, showing numerous, single, large melanosomes that are ellipsoidal and intensely melanocytic. (B) Magnification ×44 000. A keratinocyte in lesional skin of the same patient, showing smaller, less dense melanosomes that are clustered in membrane-bound groups. (C) Magnification ×6100. Bacteria present in a hair follicle of lesional skin.

Distribution LS***	Median length non-LS in µm (P25-P75)	Median length LS in µm (P25-P75)	Median diameter non-LS in µm (P25-P75)	Median diameter LS in µm (P25-P75)
Small, less melanotic, in membrane bound groups	1.2 (1.0-1.6)	0.7 (0.7-1.1)	0.4 (0.4-0.4)	0.3 (0.3-0.3)
Small, less melanotic, in membrane bound groups	1.4 (1.1-1.4)	1.2 (1.0-1.5)	0.8 (0.6-0.8)	0.7 (0.7-0.7)
Small, less melanotic, in membrane bound groups	1.1 (1.0-1.2)	1.0 (0.9-1.2)	0.8 (0.7-0.8)	0.6 (0.4-0.6)
Single smaller, less melanotic, as well as in membrane bound groups	1.5 (1.5-1.6)	1.2 (1.0-1.4)	0.7 (0.6-0.7)	0.4 (0.4-0.4)
Small, less melanotic, in membrane bound groups	1.5 (1.3-2.0)	1.0 (0.9-1.1)	0.7 (0.6-0.7)	0.5 (0.5-0.5)
Small, less melanotic, in membrane bound groups	1.6 (1.4-1.9)	1.1 (0.9-1.3)	0.7 (0.6-0.7)	0.5 (0.4-0.5)
Single smaller, less melanotic, as well as in membrane bound groups	1.9 (1.8-1.9)	1.7 (1.5-1.9)	1.0 (0.8-1.0)	1.1 (0.8-1.1)
Single smaller, less melanotic, as well as in membrane bound groups	1.8 (1.8-2.2)	1.4 (1.3-1.5)	0.8 (0.7-0.8)	0.7 (0.5-0.7)

less obvious than in patients with skin type IV to VI. In one patient with skin type V, bacteria could be identified in the sebaceous duct next to the hair follicle of the lesional skin (Figure 3C). These bacteria were not observed in the normal skin.

DISCUSSION

We showed in PMH a deficiency in the melanization of melanosomes, leading to changes in the maturation, size, number and distribution of melanosomes in lesional skin of patients with skin type V and VI. This seems to be the underlying microanatomy of hypopigmentation. In skin type III and IV, the differences between normal and lesional skin were less obvious because in those skin types, melanosomes are already smaller, less melanized and predominantly packed in aggregated groups. In one patient with skin type III, lesional skin showed 50% less melanin than normal skin. This was unexpected, because we would expect a smaller difference in total amount of melanin in this skin type (Table II). This may be a coincidence related to the effect of tanning, because the study was conducted during summertime, but it can also be explained by the fact that in EM studies only a limited part of the 2-mm

biopsy specimen is investigated and therefore may not be representative for the whole biopsy specimen.

There were no structural defects (tyrosinase functional defects can not be demonstrated by EM) nor were there disturbances in the melanosome transfer because the tips of the dendrites of the melanocytes did not show any accumulation of melanosomes and keratinocytes were not devoid of melanosomes. Furthermore, there were no defects in melanosome degradation because there were no signs of disintegrated melanosomes in the lysosomal compartments.

We conclude that a decrease in melanin synthesis, which leads to an altered distribution of melanosomes must be the explanation for hypopigmentations in PMH. This leaves us with the question in which way P. acnes can play a role in this alteration.

Possible mechanisms behind the altered distribution are 2-fold:

(1) It is known that melanosomal structure correlates with the type of melanin within (Szabo 1969, Toda *et al*. 1972, Wolff *et al*. 1974, Yamamoto *et al*. 1994, Jimbow *et al*. 1998). Jimbow *et a*l. (1998). described two types of melanosomes (large and single vs. small and aggregated). According to them, the morphology of the pigment granules, responsible for the size of the melanosomes, depends on the type of melanin formed (i.e. eumelanin or pheomelanin). In skin type I only, pheomelanin is formed, whereas in skin type VI, pheomelanin and eumelanin are formed, but the latter is present in much higher concentrations.

Studies by Del Marmol *et al*. (1996) and Smit *et al*. (1997) showed that inhibition of tyrosinase while promoting cysteine, leads to a switch from eumelanogenesis to pheomelanogenesis resulting in the formation of smaller, aggregated melanosomes. Inhibition of tyrosinase by a hypothetical factor produced by P. acnes might have the same effect. Smit *et al*. (1997) showed that a high concentration of l-tyrosine was always connected with increased pigmentation. In combination with a low l-cysteine content, there was an increase in tyrosinase activity and the highest melanin content. At high concentrations of both l-tyrosine and l-cysteine, the melanocytes showed reduced tyrosinase activity and they produced notably more pheomelanin. Strongly increased concentrations of pheomelanin were maintained in high l-tyrosine medium compared with those grown with low l-tyrosine. This was especially true for the combination with low l-cysteine showing that the l-tyrosine content of the medium strongly influences not only the eumelanin but also the pheomelanin production. They concluded that variations in the concentrations of l-tyrosine and l-cysteine could be used to regulate the melanogenetic phenotype under in vitro conditions.

(2) Wolff *et al*. (1972) showed that an important criterion for the way of melanosome distribution is the actual size of the melanosomes itself. Studies on the phagocytosis of latex beads by epidermal keratinocytes of guinea pigs showed that the mode of uptake of these melanosome like particles is size dependent. Large latex beads were incorporated singly into cells, whereas small particles were taken up in groups. As this model puts an emphasis on the active role of the keratinocyte, rather than the melanocyte, in this uptake of melanosomes, the authors suggest that the size of the individual melanosomes seemed to be the decisive factor that determines the distribution of pigment organelles.

Minwalla *et al*. (2001), however, showed that in a co-culture of keratinocytes and melanocytes, melanosome size does not correlate with the ultimate pattern of distribution within the keratinocyte. Studies showed that recipient melanosomes, regardless of skin type, are predominantly distributed individually by keratinocytes from dark skin and in membrane-bound clusters by those from light skin. Melanosome size was not related to whether the melanosomes were distributed individually or clustered. The authors suggested that regulatory factors within the keratinocyte determine recipient melanosome distribution patterns.

A hypothetical factor produced by P. acnes might influence such regulatory factors within the keratinocyte that determine recipient melanosome distribution patterns as described by Minwalla *et al*. (2001).

Further research on the l-tyrosine and l-cysteine content of the skin and the role of regulatory factors in melanosome distribution in PMH is apparently necessary.

With this study, the findings of Guillet *et al*. (1988) who studied only two patients were confirmed; in addition, the dynamics of PMH on the ultrastructural level were further elucidated. We conducted a more specified and detailed study with a larger patient population and a greater diversity in skin types, also performing (semi)quantitative measurements of the melanosome size. Like Kumarasinghe *et al*. (2006) who also observed PMH in Chinese, Mongoloid and Indian type people we disagree with Guillet et al.'s conclusion that the hypopigmentations are caused by a 'melting' of genes of white and black parents, because we also diagnosed PMH in patients of other ethnicities and with a skin colour ranging from skin type III to VI.

The results presented facilitate a sharper delineation of PMH from other hypopigmentation disorders like PV and PA. The keynote findings in PV are the presence of yeast cells and hyphae, degenerative altered melanocytes (Galadari *et al*. 1992, Breathnach *et al*.1975) and partial blocking of the transfer of melanosomes (Charles *et al*. 1973), all these phenomena lacking in PMH. The keynote findings

in PA are characteristics of dermatitis, also lacking in PMH (Urano-Suehisa *et al*. 1985). Electron microscopic studies in PA are rare, although Zaynoun *et al*. (1983) conducted electron microscopic studies in what they called 'extensive pityriasis alba' (EPA). However, in our view, their study is not valid for comparison, because we believe EPA to be identical with PMH (Relyveld *et al*. 2006).

The histological and electron microscopic findings presented assist in further defining the unique clinical picture of PMH. We believe further investigations are necessary to further unravel the pathogenesis of PMH and to make a connection between our present ultrastructural findings and our hypothesis of a microbial factor being the cause of the hypopigmentations.

GN Relyveld
M Kingswijk
JB Reitsma
HE Menke
JD Bos
W Westerhof

Benzoyl peroxide/clindamycin/UVA is more effective than fluticasone/UVA in progressive macular hypomelanosis: a randomized study. *J Am Acad Dermatol*. 2006;55:836-843

6 BENZOYL PEROXIDE/ CLINDAMYCIN/UVA IS MORE EFFECTIVE THAN FLUTICASONE/ UVA IN PROGRESSIVE MACULAR HYPOMELANOSIS: A RANDOMIZED STUDY

ABSTRACT

Introduction: There is no effective treatment for progressive macular hypomelanosis. Recent findings indicate that *Propionibacterium acnes* may play a role in the pathogenesis. We sought to compare the effectiveness of antimicrobial therapy with anti-inflammatory therapy in patients with progressive macular hypomelanosis.

Patients and Methods: A total of 45 patients were randomized to a within-patient left-right comparison study of benzoyl peroxide 5% hydrogel/clindamycin 1% lotion in combination with UVA irradiation versus fluticasone 0.05% cream in combination with UVA irradiation. Repigmentation was determined by photometric measurements of changes in skin color and by patient and dermatologist assessment using before and after photographs.

Results: Benzoyl peroxide 5% hydrogel, clindamycin 1% lotion, and UVA led to better repigmentation than fluticasone 0.05% cream in combination with UVA irradiation in all measurements. (Photometric measurements $P = .007$, patient assessment $P < .0001$, and dermatologist assessment $P < .0001$.) There was difficult objective color measurement. Therefore, subjective assessment has important additional value. Right-left comparisons have certain inherent limitations.

Conclusion: Antimicrobial therapy in conjunction with light was more effective in repigmentation in patients with progressive macular hypomelanosis than a combination of anti-inflammatory therapy and light.

INTRODUCTION

Progressive macular hypomelanosis (PMH) is a skin disorder of the trunk, rarely extending to the neck/head region, proximal extremities, or both, characterized by ill-defined, nummular, symmetrically localized hypopigmented macules. In the majority of patients, a hypopigmented area is present on the front and the back of the trunk that seems to originate from confluence of the macules (Guillet *et al*. 1988, 1992, Lesueur *et al*. 1994, Menke *et al*. 1997, 1998). Diagnostic criteria include characteristic clinical features as described above and the presence of red follicular fluorescence in hypopigmented spots that is absent in adjacent normal skin. Pityriasis versicolor is excluded by negative potassium hydroxide test results of epidermal scrapings. PMH might be more common in tropical and subtropical countries, but prevalence studies are scarce. In 1994, Lesueur *et al*. diagnosed 121 cases of PMH during a screening for leprosy among 511 patients in the French West Indies (Martinique). Guillet *et al*. 1992 diagnosed 150 new cases per year in their dermatology clinic in Martinique. Little is known about the origin and pathogenesis of PMH. Ultrastructural studies conducted by Guillet *et al*. in 1988 showed stage IV single melanosomes in nonlesional skin and small type stage I to III aggregated melanosomes in lesional skin of patients from mixed (Negroid-Caucasoid) background.

In 2004, Westerhof *et al*. proposed that PMH is caused by *Propionibacterium acnes*. This suggestion was based on the observation that illumination of the hypopigmented spots with a Wood's lamp in a dark room produces a red follicular fluorescence, which is absent in normal adjacent skin. This was further substantiated by culturing *P acnes* from pilosebaceous ducts of lesional skin. The hypothesis was formulated that *P acnes* produces a factor that interferes with melanogenesis, leading to hypopigmented macules. Eliminating *P acnes* with topical antibacterial therapy, such as in acne, could, therefore, improve repigmentation in patients with PMH. A combination therapy of clindamycin lotion and benzoyl peroxide hydrogel would be recommended because research has shown that in patients with mild to moderate acne this combination has significantly better results than either of the two components alone. Furthermore, benzoyl peroxide combined with topical antibiotics reduces the risk that resistant strains of *P acnes* develop (Leyden *et al*. 2001, Leyden 2003, Ellis *et al*. 2001). Another view on the pathogenesis, based on our histologic examination showing mild perifollicular lymphocytic infiltration (Westerhof *et al*. 2004), is that hypopigmentation is secondary to an inflammatory

process, although there are no clinical signs of inflammation in PMH. This would suggest that anti-inflammatory agents such as topical corticosteroids could be a possible treatment.
We conducted a trial to examine whether antibacterial treatment is more effective in repigmentation than anti-inflammatory treatment in patients with PMH.

METHODS

We performed a within-patient, left-right randomized trial comparing benzoyl peroxide/clindamycin in combination with UVA (bcUVA) with fluticasone in combination with UVA (fUVA) in patients with PMH. The medical ethical committee of the hospital approved the study protocol and written consent was obtained from all patients.

Patients

Patients with PMH between 16 and 55 years of age were eligible for inclusion. The diagnosis of PMH was based on clinical findings including the presence of red follicular fluorescence in the hypopigmented spots when illuminated with a Wood's lamp in a dark room. Patients were excluded if they: had positive potassium hydroxide test results; were sensitive to any of the study medication ingredients or sunlight; were treated with chemical peeling or other treatments that could cause scaling of the trunk; or were pregnant or lactating. In addition, any previous treatment for PMH or any antibacterial treatment (both local and systemic) had to be stopped at least 3 months before study entry.

Interventions

Patients received instructions for daily application of benzoyl peroxide 5% hydrogel at night and clindamycin 1% lotion in the morning on one side (antibacterial treatment) and fluticasone cream 0.05% at night on the other side (anti-inflammatory treatment). A computerized randomization program was used by the treating physician to decide which side received which therapy. Patients and the treating physician were not blinded for treatment allocation.
Patients applied the medication themselves during a period of 14 weeks. During this period, patients exposed both sides to UVA light for 20 minutes 3 times a week. For this purpose they received a half-body solarium (HB 406, Philips, Eindhoven, the Netherlands) and were instructed to sit at a distance of 55 cm in front of the

solarium. After 20 minutes at this distance, the effective flux on the skin (H-IECeff) is 233 J/m^2.

All treatments were stopped after 14 weeks, but patients were instructed to stay out of the sun for an additional period of 12 weeks. If sun exposure could not be avoided, patients were advised to apply a sunscreen with a protecting factor of at least 30 every 2 hours.

Objective skin color measurements

The primary outcome in our trial was the difference in repigmentation between bcUVA- and fUVA-treated areas as measured by the colorimeter. We measured skin color at baseline; after 2, 6, 10, and 14 weeks of treatment; and after a period of 12 weeks without treatment (t = 26 weeks), using a spectrocolorimeter (Microflash 200d, Datacolor, Lawrenceville, New Jersey). This colorimeter uses a system devised in 1976 by the Commission International de l'Éclairage (CIE). Various investigators have extensively used the technique to quantitatively compare erythema, pigmentation, and skin color (Shriver and Parra 2000, Alaluf *et al*. 2002, Wagner *et al*. 2002). It transforms a reflectance spectrum R (λ) into 3 values: L^*, a^*, and b^*. L^* represents the lightness of the spectrum and varies from 0 for a black object to 100 for a white object, a^* represents green (negative values) and red (positive values), and b^* represents blue (negative values) and yellow (positive values). Total epidermal melanin content primarily determines L^* values in human skin, whereas melanosome size also has a significant but more subtle influence on L^* values (Alaluf *et al*. 2002, Wagner J *et al*. 2002). Because PMH lesions reveal shortage of epidermal melanin (own observations in histologic and electron microscopic investigations), we focused on comparing L^* values. We measured two hypopigmented lesions on each side. Each lesion was measured twice, and the average value was used in the calculations. The diameter of the measured lesion had to be at least 1.5 cm to be larger than our instrument's measuring diameter (0.9 cm). Normal adjacent skin was measured in the same way. Measurements were always done at the same spot and under the same external conditions by the same researcher. The researcher conducting the skin color measurements was blinded for treatment allocation.

Subjective assessment by patients, dermatologists, and study assistant

Images of the trunk were taken at each visit using a camera (Dynax 5xi, Minolta, Tokyo, Japan). Conditions were standardized throughout the study, including the same room, lighting conditions, and distance between camera and patient. Each patient scored his or her treatment success by comparing before (t = 0) with after (t =

14 and t = 26) photographs. Two dermatologists and one study assistant, who were blinded for the assigned treatment, independently scored the same photographs. Repigmentation relative to the baseline situation was scored on a 5-point scale: ++ = total repigmentation, + = moderate repigmentation, 0 = no change, – = moderate worsening, and –– = severe worsening. We took the mean of the subjective scores of the two dermatologists and the assistant from each patient as the final score.

Adverse effects

At each visit, patients filled in questionnaires about compliance, side effects, pregnancy or lactation, and whether they had started any other medication.

Sample size determination

Based on personal observations, we estimated that treatment with fUVA therapy would be moderately effective in about 40% of the patients. To demonstrate a clinically relevant improvement to 70% would require a sample size of 42 patients (2-sided significance level of 5% and a power of 80%).

Statistical analysis

We defined ΔL^* as the difference between L^* values for lesional and normal skin. ΔL^* values for each side were determined at baseline (t = 0), after 14 weeks of treatment (t = 14), and after 12 additional weeks without treatment (t = 26). Improvement in ΔL^* values from baseline to t = 14 were calculated for each treatment side by subtracting these scores. Improvements in repigmentation were then compared between the fUVA- and the bcUVA-treated side using a paired-samples *t* test. A similar analysis was done to test for differences in repigmentation at t = 26 weeks. The subjective assessment scores of the patients, dermatologists, and assistant were analyzed by comparing the scores of both sides within each patient. The McNemar test was used to compare the number of patients in which the bcUVA side was judged better with the number of patients with higher scores for the fUVA-treated side.

RESULTS

Baseline characteristics

We invited 184 patients with PMH for a screening visit, of whom 52 replied. All these patients met our inclusion criteria and were randomized. However, 7 female patients withdrew after 1 week of treatment, leaving 45 patients in our study. One female patient discontinued her benzoyl peroxide/clindamycin treatment after 6 weeks because of an adverse effect to benzoyl peroxide but stayed in the

analysis (Figure 1). Demographics and baseline characteristics of the 45 patients are presented in Table I.

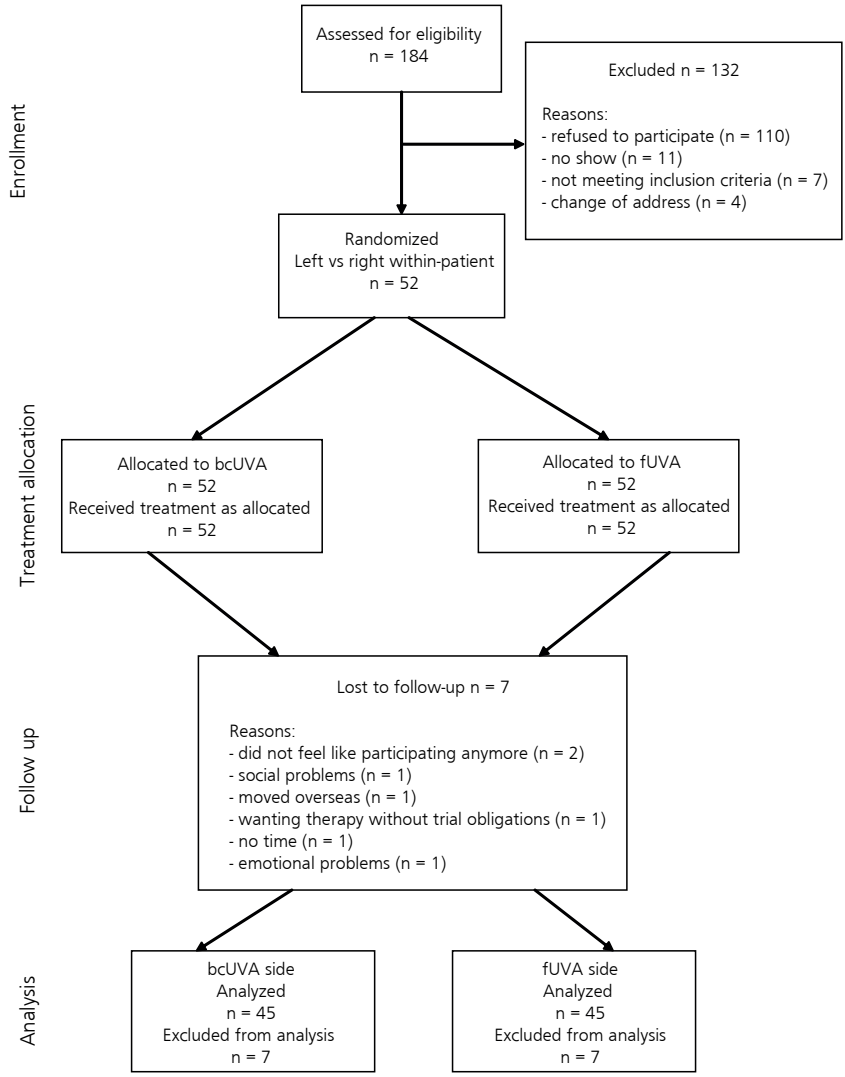

Figure 1 Flow of participants in study. bcUVA, Benzoyl peroxide 5% hydrogel and clindamycin 1% lotion in combination with UVA phototherapy; fUVA, fluticasone cream in combination with UVA phototherapy.

Table I Baseline characteristics of the study population

Characteristics	n = 45
Female, No. (%)	36 (80)
Mean age, y ± SD	27.5 ± 8.7
Skin type,* No. (%)	
II	7 (16)
III	9 (20)
IV	8 (18)
V	17 (38)
VI	4 (9)
Median duration of PMH in y	5
P25-P75	3-10
Former treatment,† No. (%)	
PUVA	1 (2.2)
UVB	5 (11)
Fluticasone + UVB	1 (2.2)
Fluticasone + benzoyl peroxide + UVB	1 (2.2)
fUVA	2 (4.4)
Antimycotics	20 (44)
Antimycotics + UVB	1 (2.2)
No treatment	14 (31)
Mean L* values at baseline measurements ± SD	
Normal skin adjacent bcUVA	52.8 ± 8.3
Normal skin adjacent fUVA	52.8 ± 8.3
bcUVA Side	56.1 ± 8.7
fUVA Side	56.1 ± 8.3
Full-size table	

bcUVA, Benzoyl peroxide 5% hydrogel and clindamycin 1% lotion in combination with UVA phototherapy; fUVA, fluticasone cream in combination with UVA phototherapy; PMH, progressive macular hypopigmentation; PUVA, psoralen and UVA radiation; P25-P75, 25th to 75th percentile. * According to Fitzpatrick. † Patients could be in more than one category.

Skin color measurements

After 14 weeks of treatment, both the treated sides became darker than normal skin, but this effect was more pronounced on the bcUVA side. After 12 weeks without treatment, the antibacterial (bcUVA)-treated side had the same degree of

pigmentation as normal skin whereas the anti-inflammatory (fUVA)-treated side was lighter than normal skin. The mean difference in L values between the two sides after 26 weeks was 0.9 points and highly significant (P = .007, 95% confidence interval 0.3-1.5). Although the skin on the bcUVA side remained evenly pigmented, hypopigmented macules reappeared on the fUVA side (Figure 2).

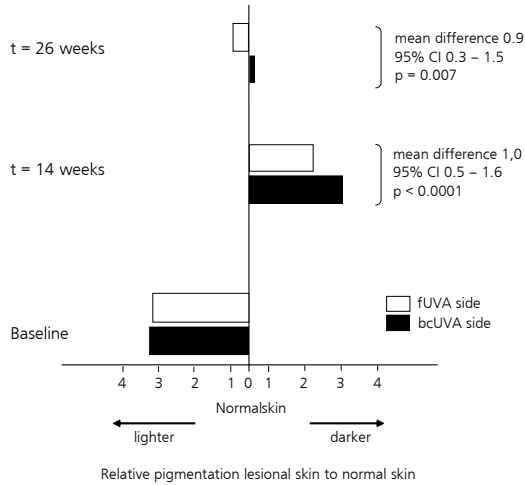

Figure 2 Measurements after 14 weeks of daily treatment (t = 14 weeks) and after 12-week period without any treatment (t = 26 weeks). bcUVA, Benzoyl peroxide 5% hydrogel and clindamycin 1% lotion in combination with UVA phototherapy; CI, confidence interval; fUVA, fluticasone cream in combination with UVA phototherapy.

Assessment scores

The patients, dermatologists, and assistant scored the bcUVA-treated side higher than the fUVA-treated side (Figure 3). This difference was highly significant after 14 weeks of treatment (P < .0001 for patients, P < .0001 for dermatologists and assistant). At the end of follow-up, 62% of the patients judged their bcUVA-treated side as totally repigmented but only 13% of the patients gave such a score to their fUVA-treated side (P < .0001). The dermatologists scored 62% of the bcUVA-treated sides and 22% of fUVA-treated sides as totally repigmented at the end of follow-up (P < .0001) (Figure 4A,B).

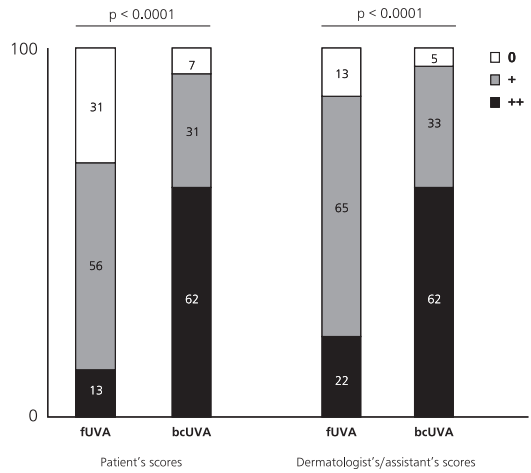

Figure 3 Distribution of long-term treatment effect (at t = 26 weeks consisting of 14 weeks of treatment and 12 weeks without) as scored by patients, two external dermatologists, and assistant (all 3 masked for treatment assignment). Numbers indicate percentages of patients and dermatologists/assistant giving particular score. bcUVA, Benzoyl peroxide 5% hydrogel and clindamycin 1% lotion in combination with UVA phototherapy; fUVA, fluticasone cream in combination with UVA phototherapy; ++, total repigmentation; +, moderate repigmentation; 0/–, no repigmentation/no worsening of hypopigmentations.

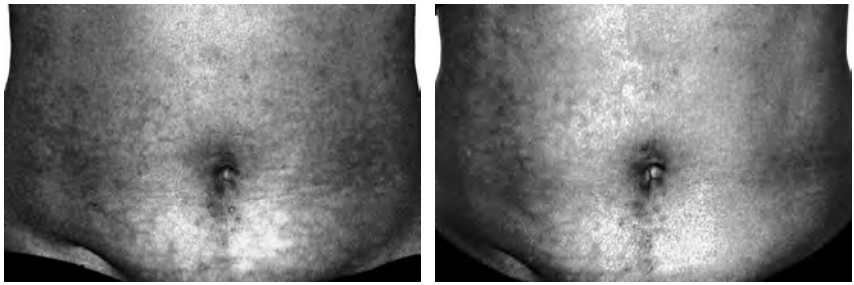

Figure 4 (A) Patient (29-year-old woman) with progressive macular hypomelanosis (skin type VI) at baseline. (B) Same patient after 26 weeks. Left side of trunk was treated with benzoyl peroxide 5% hydrogel and clindamycin 1% lotion in combination with UVA phototherapy and right side with fluticasone cream in combination with UVA phototherapy.

Adverse effects

Most adverse effects were mild and followed anticipated patterns (Table II). More patients reported cutaneous side effects with antibacterial than with corticosteroid therapy (71% vs 24%, $P < .0001$). The incidence of side effects decreased after

Table II Number (percentage) of patients who reported one or more local side effects

	Benzoyl peroxide/clindamycin n = 45	Fluticasone n = 45
Dry skin	27 (60)	10 (22)
Itching	14 (31)	3 (6.7)
Scaling	13 (29)	5 (11)
Erythema	6 (13)	1 (2.2)
Burning sensation	4 (8.9)	0
Edema	1 (2.2)	0
Total no. of patients who reported side effects	32 (71)	11 (24)*
Full-size table		

Patients could report more than one side effect. *χ2, 1 df, P less than .0001.

the second week of both treatments. After the sixth week, only 4 (9%) patients mentioned side effects on the bcUVA side and 3 (7%) patients mentioned side effects on the fUVA side.

DISCUSSION

Antibacterial therapy was more effective than anti-inflammatory therapy in the treatment of PMH. Antibacterial therapy led to better repigmentation as indicated by darker objective skin measurements and, more importantly, by higher scores for treatment success by both patients and dermatologists.

Our results lend support to the hypothesis of Westerhof *et al*. in 2004 that *P acnes* is causally related to PMH. Although we did not measure *P acnes* colonies in the treated skin objectively, subjective Wood's lamp investigations suggested a faster and complete elimination of *P acnes* in the treated area on the bcUVA side, compared with a slow and limited elimination on the fUVA side.

Repigmentation

At the end of follow-up, the mean difference in repigmentation (L^* value) between the two treated sides was 0.9 points and highly significant. L^* values differentiate between lightness (higher scores) and darkness (lower scores), and correlate with the melanin content of the skin (Wagner *et al*. 2002). The difference might seem small, but such a difference in skin color is directly noticeable. This is confirmed by the subjective judgments given by both patients and physicians to the side treated

with antimicrobial therapy. The exact duration of the effect after treatment is not yet known, but 3 months after cessation of therapy the effect was still visible.

Strengths and limitations

Several design characteristics of our study deserve further attention. The within-patient comparison (left-right randomization) is a statistically efficient design as each patient serves as his or her own control. Potential problems of carryover effects are not an issue because both treatments work locally. An additional benefit of the within-patient parallel comparison is that the effect of both treatments can be judged simultaneously in each patient using one photograph. Because each patient receives both treatments, it avoids problems caused by changes in patient behavior after disappointment or favorable expectations if patients receive only one treatment. We paid particular attention to achieve objective measurements for repigmentation. The CIE $L^*a^*b^*$ system has been extensively tested (Shriver *et al.* 2000, Alaluf *et al.* 2002) and used in several other trials of skin disorders leading to discoloration. However, objective color measurement of a particular lesion is not without problems, especially when repigmentation is patchy. Subjective assessment of the overall treatment result has, therefore, important additional value because it will take into account whether repigmentation is uniform and whether the affected skin looks different from the normal adjacent skin in aspects other than hypopigmentation. In our trial both objective and subjective outcomes pointed in the same direction: repigmentation was more successful and more uniform on the side treated with antibacterial therapy. In our trial we combined both treatments with UVA radiation. Because we found a highly significant difference in repigmentation between the two interventions, it virtually excludes that UVA radiation is the sole factor responsible for treatment success. We believe that UVA does not treat the underlying cause but speeds repigmentation after the antibacterial treatment has removed the underlying inhibitive factor. This view is supported by our observation that various patients treated with benzoyl peroxide/clindamycin therapy but without UVA irradiation achieve total repigmentation. Furthermore, exposure to sun (consisting also of UVA rays) does not improve the skin lesions in patients with untreated PMH (personal observations).

Recommendations for practice and research

To our knowledge, no other prospective studies on the treatment of PMH have been done. Dermatology textbooks consider the disease as noncurable, and give little

information on the effectiveness of any treatment (Menke *et al*. 1998, Ortonne *et al*. 2003).

The therapeutic success of the bcUVA combination is probably related to the elimination of *P acnes*. We did not investigate the optimal dose for benzoyl peroxide/clindamycin, other routes of administration, or whether other types of antibiotics are equally effective. We chose a combination therapy of clindamycin lotion and benzoyl peroxide hydrogel because research has shown that in patients with mild to moderate acne this combination had significantly better results than either of the two components alone. Furthermore, it is known that benzoyl peroxide combined with topical antibiotics reduces the risk that resistant strains of *P acnes* develop (Leyden *et al*. 2001, Leyden 2003, Ellis *et al*. 2001). Because formulations that combine both benzoyl peroxide and clindamycin were not available on the Dutch market at the time of the trial, we applied the products separately.

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Progressive and extensive pityriasis alba: same disease, different names? *J Eur Acad Dermatol Venereol*. 2006;20(10):1363-1364

7 PROGRESSIVE AND EXTENSIVE PITYRIASIS ALBA: SAME DISEASE, DIFFERENT NAMES?

Editor

We read with much attention the article by di Lernia and Ricci (2005) as for many years now we have been greatly interested in the pigmentary disorder called progressive and extensive hypomelanosis and which we indicate as progressive macular hypomelanosis, according to Guillet *et al*. (1988). We have the following comments:

(1) In 1987 Borelli (1987) published an article about a new disorder with hypopigmentations on the trunk, which he named 'cutis trunci variata'. Comparison of his clinical description with that of Guillet *et al*. (1988) suggests that the same disorder is described in these two publications. Therefore, it appears that Borelli, and not Guillet *et al*. was the first to publish this disorder.

(2) Clinical description and light microscopy suggest that extensive pityriasis alba described by Zaynoun *et al*. (1983) and progressive macular hypomelanosis described by Guillet *et al*. (1988) might indeed be the same disorder. However, we want to point out that the electron microscopic findings are different. The electron microscopic investigations conducted by Zaynoun *et al*. (1983) on nine patients with extensive pityriasis alba showed reduced melanocytes and those present contained fewer and smaller melanosomes. Furthermore, a reduction in the density of functional melanocytes in the affected areas without any change in cytoplasmic activity was observed. Melanosomal transfer to keratinocytes was generally not disturbed. They concluded that the hypopigmentation might thus be primarily a result of the reduced numbers of active melanocytes and a decrease in number and size of melanosomes in the affected skin. Histological investigations by Guillet *et al*. (1988) showed a slight reduction of melanin granules in the basal cell layer with a variable decrease of melanin transfer to keratinocytes. Ultrastructural investigations showed a switch from stage IV single melanosomes (these are the types of melanosomes normally seen in black skin) in the healthy looking skin to small type stage I–III aggregated melanosomes (these are the types of melanosomes normally seen in white skin) in the hypopigmented spots.

(3) If extensive pityriasis alba is indeed identical to progressive macular hypomelanosis, we advise that the first name is abandoned. The term pityriasis alba is generally accepted to indicate a disorder with minimal eczematous characteristics (Weedon 2002). These are not present in progressive macular hypomelanosis. Extensive pityriasis alba is therefore a misnomer.

(4) We have recognized hundreds of patients of both sexes and different ethnicities with progressive macular hypomelanosis in the Netherlands since the early eighties of the last century. Since 1989 we have presented our findings at several international and Dutch meetings and have also published these findings. (References are available on request).

(5) In 2004 we proposed the hypothesis that progressive macular hypomelanosis is caused by *Propionibacterium acnes* (*P. acnes*), based on clinical and microbiological Investigations (Westerhof *et al*. 2004). Studies on PMH patients by Wood's lamp in a dark room showed red follicular fluorescence in lesional skin, which was not present in normal skin. Red fluorescence is also seen in patients with acne and represents excitation of porphyrins produced by *P. acnes* bacteria. Furthermore, bacterial cultures of hair follicles in lesionial skin in seven out of eight patients with PMH were positive for *P. acnes* while bacterial cultures of non-lesional skin in the same patients showed no bacterial growth. This proves that PMH is a distinct disease entity with a separate aetiopathogenesis. Based on these observations we soon hope to publish the results of a clinical trial concerning the treatment of the disease, which so far is intractable.

8 ALGEMENE DISCUSSIE, SAMENVATTING EN CONCLUSIES

Algemene discussie, samenvatting en conclusies

In 1987 verscheen het eerste artikel over een huidziekte met symmetrisch gelokaliseerde, gehypopigmenteerde maculae op de romp die wat betreft morfologie, beloop en behandeleffect verschilde van andere dermatosen die gepaard gaan met hypopigmentatie (Borelli 1987). In 1988 werd de naam Progressieve Maculaire Hypomelanose (PMH) aan deze ziekte gegeven (Guillet *et al*. 1988). Tot voor kort was er slechts spaarzaam informatie over dit ziektebeeld in de literatuur terug te vinden en in de praktijk bleek vaak dat deze entiteit niet als nieuw gezien werd, maar als een (resttoestand van) andere, bekende huidaandoening zoals pityriasis versicolor.

Door de jaren heen kwamen er steeds meer beschrijvingen van vergelijkbare huidbeelden met name "Creole dyschromia" (Lesueur *et al*. 1994), "idiopathic multiple large macule hypomelanosis" (Fitzpatrick 1996), "nummular and confluent hypomelanosis of the trunk" (Menke *et al*. 1997), echter allen met een andere naam en systematisch onderzoek ontbrak helaas.
Met de studies die door ons zijn uitgevoerd, hebben wij getracht op systematische wijze vorm te geven aan deze relatief nieuwe entiteit. Wij hebben ons hiervoor verdiept in de pathologie, epidemiologie, oorzaak, kliniek en behandeling van PMH.

Hoofdstuk 1 is een inleidend hoofdstuk, waarin wordt ingegaan op de historische achtergrond en terminologie van PMH. Het hoofdstuk betreft een samenvatting van wat er bekend was over het ziektebeeld ten tijde van het begin van onze studies.

Hoofdstuk 2 geeft een beschrijving van de bevindingen bij histologisch en microbiologisch onderzoek bij PMH. Deze studie is verricht naar aanleiding van waarnemingen tijdens het lichamelijk onderzoek, waarbij opviel dat bij belichting van de huid met de Wood's lamp in een donkere kamer, rode, folliculaire fluorescentie aanwezig was in de aangedane huid. Deze fluorescentie was afwezig in de follikels van de aangrenzende normale huid. Dit deed vermoeden dat het hier om een porfyrine producerende bacterie ging, b.v. *Corynebacterium minutissimum* of een *Propionibacterium* soort gezien het feit dat rode folliculaire fluorescentie een van de karakteristieken van deze bacteriën is.

Wij hebben bij 8 patiënten biopten genomen van de follikels van lesionale en aangrenzende, normale huid en van de inter-folliculaire, lesionale en aangrenzende, normale huid.

Histologisch onderzoek van de biopten van de folliculaire, lesionale huid liet bij alle 8 patiënten Gram positieve bacteriën zien in de afvoergangen van de talgklieren. In de follikels van de normale huid en in de inter-folliculaire huid (zowel lesionaal als normaal) werd dit niet waargenomen. Conventionele kweekmethode liet groei zien van *P. acnes* bacteriën in 7 van de 8 biopten van de follikels van de lesionale huid. In alle andere biopten waren de *P. acnes* kweken negatief.

Geconcludeerd werd dat er een relatie lijkt te zijn tussen de aanwezigheid van *P. acnes* en de gehypopigmenteerde mauclae. Verder werd gespeculeerd dat de bacterie mogelijk een depigmenterende stof produceert die de pigmentsynthese hindert.

In **Hoofdstuk 3** wordt nader ingegaan op het type *Propioni* bacterie dat wordt gezien bij PMH. *P. acnes* speelt een rol bij het ontstaan van acne. Echter, wij hebben in een eerdere studie (zie hoofdstuk 2) gesuggereerd dat deze bacterie mogelijk ook een belangrijke rol speelt bij het ontstaan van PMH. Opvallend is dat over het algemeen patiënten die zich presenteren met PMH geen acne hebben. Dit heeft geleid tot de hypothese dat mogelijk een ander subtype van *P. acnes* betrokken is bij het ontstaan van PMH.

Om dit verder uit te zoeken hebben wij een studie uitgevoerd waarbij wij biopten hebben afgenomen van een rode, fluorescerende follikel van de lesionale huid van 14 PMH patiënten en van een rode, fluorescerende follikel ter plaatse van een acne laesie van 10 acne patiënten. De biopten werden vervolgens uitgestreken op bloedagar platen en provisorische, conventionele identificatie werd toegepast. Vervolgens hebben wij van iedere plaat 1 isolaat verder geanalyseerd met behulp van de DNA vingerafdruk methode: amplified fragment length polymorfism (AFLP). Ter bevestiging van onze AFLP resultaten hebben wij vervolgens 16S rRNA gene sequencing analyses uitgevoerd van isolaten afkomstig uit de verschillende DNA groepen uit de AFLP analyses. Deze isolaten hebben wij vergeleken met de nucleotide reeks van referentiestam *P. acnes*: ATCC 6919. Om eventuele "menging" van isolaten uit te sluiten hebben wij vervolgens opnieuw bacterie isolaten gekweekt

van 3 acne patiënten en 3 PMH patiënten. Wij hebben een maximum van 10 isolaten per patiënt gekweekt. De AFLP analyse is opnieuw voor alle gekweekte isolaten uitgevoerd.

Alle provisorisch, conventioneel geïdentificeerde isolaten bleken *P. acnes* bacteriën te zijn. De AFLP analyse resulteerde in 3 verschillende DNA groepen. Groep 1 betrof isolaten van 8 acne patiënten en 6 PMH patiënten. Deze DNA groep liet een gelijkenis tussen de 55 en 100% zien met de *P. acnes* referentie stam. Dit suggereert dat het gaat om identieke of verschillende stammen van hetzelfde species. Isolaten uit DNA groep 2 (afkomstig van 2 acne patiënten) lieten een gelijkenis tussen de 30 en 55% zien met de referentie stam *P. acnes*. Dit suggereert dat het een gerelateerde species van *P. acnes* betreft, mogelijk een subspecies. Isolaten uit groep 3 (afkomstig van 8 PMH patiënten) vormden een duidelijk op zichzelf staande DNA groep met een gelijkenis van minder dan 30% met de referentie *P. acnes* stam. Ook de gelijkenis met referentiestammen *P. granulosum*, *P. avidum* en *P. propionicus* was minder dan 30%. Dit suggereert een andere species op basis van de lage procentuele homologie. 16S rRNA gene sequencing analyse van 2 isolaten afkomstig uit DNA groep 1, van 1 isolaat afkomstig uit DNA groep 2 en van 3 isolaten afkomstig uit DNA groep 3 liet het volgende zien: isolaten afkomstig uit groep 1 lieten geen verschillen met de referentiestam zien. Het isolaat afkomstig uit groep 2 liet een enkel nucleotide verschil zien op positie 827 met de referentiestam. Twee isolaten uit groep 3 lieten een enkel nucleotide verschil zien op positie 1243 met de referentiestam en 1 isolaat uit groep 3 liet twee nucleotide verschillen zien op positie 712 en positie 1243. De resultaten van de tweede AFLP analyse kwamen overeen met onze vorige bevindingen en "menging" van isolaten kon daarmee worden uitgesloten. Aanvullende biochemische testen van de bacteriën afkomstig van de drie verschillende DNA groepen bevestigden onze AFLP bevindingen, echter het resistentiepatroon van deze bacteriën was exact hetzelfde.

Gezien de kleine verschillen die gevonden zijn met de 16S rRNA gene sequencing analyse en biochemische testen en het vergelijkbare resistentiepatroon tussen de verschillende DNA groepen, hebben wij geconcludeerd dat wij mogelijk te maken hebben met een tot nog toe ongedefinieerd *Propionibacterium* species. Het feit dat de bacteriën uit DNA groep 3 alleen maar gevonden zijn in PMH patiënten en niet bij acne patiënten, heeft ons doen concluderen dat er een relatie is tussen de groep III *Propioni* bacteriën en PMH.

Hoofdstuk 4 geeft een beschrijving van PMH binnen de patiëntenpopulatie in de Stichting Nederlands Instituut voor Pigmentstoornissen. Ondanks verscheidene beschrijvingen van PMH in de literatuur, lijkt er geen overeenstemming te zijn betreffende de karakteristieken van deze ziekte.

Om een beter inzicht te krijgen in het klinische beeld en het natuurlijk beloop van PMH, hebben wij een enquête gehouden die bestond uit vragen over het huid type van de patient, vragen betreffende de start van de ziekte, de familie anamnese, de klinische kenmerken, de symptomen van de aandoening, de progressie en eventuele eerdere behandelingen. Verder hebben wij gevraagd naar de medische voorgeschiedenis van patiënten, om na te gaan of er relaties mogelijk waren met andere ziekten. De enquete is verstuurd aan 152 PMH patienten uit de SNIP en 101 patiënten hebben de enquete volledig ingevuld geretourneerd. De uitslagen van deze enquête zijn vervolgens vergeleken met de beschrijvingen van het klinisch beeld in de literatuur om een compleet overzicht te geven.

PMH bestaat uit symmetrisch gelokaliseerde, gehypopigmenteerde, niet-schilferende maculae op de romp. Hoewel de meningen in de literatuur verschillen als het gaat om het beloop, lijkt de aandoening progressief te zijn. Meestal begint het op de romp en breidt de ziekte zich later verder uit over de romp en bij een beperkt deel van de patiënten naar het gezicht en naar het proximale deel van de extremiteiten. Vaak treedt confluering op van de maculae op de romp. Spontane remissie treedt waarschijnlijk op na adolescentie, gezien het feit dat PMH bij mensen van middelbare leeftijd en ouderen, voor zover bekend, niet voorkomt. Indien spontane remissie na therapie optreedt, komen de maculae vaak terug op precies dezelfde plaatsen. PMH wordt met name gezien bij adolescenten en jong volwassenen. De verdeling man : vrouw blijft onduidelijk, hoewel de meeste auteurs de aandoening vooral bij vrouwen zien, wat overeenkomt met de verdeling in onze patiëntenpopulatie. Het merendeel van de patiënten heeft huidtype III tot en met VI en is van gemengde (vooral caucasisch-negroide) afkomst, echter Aziaten en Mongolen zijn ook beschreven. Bij de meeste patienten werd de diagnose pityriasis versicolor gesteld en patienten werden vaak behandeld met lokale antimycotica. Niet onverwacht was dat bij geen van de patiënten deze behandeling een goed resultaat heeft gehad.

Gebaseerd op de huidige beschikbare informatie betreffende PMH hebben wij geconcludeerd dat onze studie een zo compleet mogelijke en overzichtelijke

beschrijving geeft van het klinisch beeld en het beloop van PMH. Verder kunnen de uitkomsten van deze studie dienen als een richtlijn voor artsen bij het diagnosticeren van PMH bij patiënten met hypopigmentaties.

In **Hoofdstuk 5** worden de electronenmicroscopische bevindingen bij PMH beschreven. In deze studie zijn wij op zoek gegaan naar een verklaring voor de hypopigmentaties met als doel een aanwijzing te krijgen over het ontstaansmechanisme.

Bij 8 PMH patiënten met huidtype III tot en met VI hebben wij 2 mm biopten afgenomen van de gehypopigmenteerde en aangrenzende normale huid. Deze biopten zijn door middel van electronenmicroscopie beoordeeld en de bevindingen zijn met elkaar vergeleken.

Uit deze studie is gebleken dat bij patiënten met huidtype V en VI de melanosomen die gevormd worden door de melanocyten in de lesionale huid van PMH patiënten beschreven kunnen worden als kleinere, stadium I tot III geaggregeerde melanosomen, terwijl de melanosomen in de normale, aangrenzende huid beschreven kunnen worden als grotere, stadium IV, enkelvoudige melanosomen. Stadium I t/m III melanosomen worden normaliter gezien in de blanke huid (huidtype I tot en met IV). Stadium IV melanosomen worden gezien in de getinte en donkere huid (huidtype V en VI). De overdracht van melanosomen van de melanocyt naar de keratinocyt lijkt ongestoord. Er is geen ophoping van melanosomen in de dendrieten waarneembaar. Ook de functie van de melanocyt lijkt ongestoord, gezien het feit dat alle precursors van melanosomen aanwezig zijn in de melanocyt.

Wij brengen de hypothese naar voren dat er mogelijk een factor door *P. acnes* wordt geproduceerd die de vorming van stadium IV melanosomen hindert. Verscheidene mechanismen kunnen hieraan ten grondslag liggen: a) door inhibitie van de tyrosinase activiteit en toename van de L-cysteïne concentratie onder invloed van de hypothetische factor treedt een switch op van eumelanogenese naar feomelanogenese, die resulteert in kleinere, geaggregeerde melansomen (stadium I tot III) en lichte huidskleur. In dit voorstel is het doel van de factor de melanocyt; b) Recente studies hebben daarnaast laten zien dat factoren in de keratinocyten bepalen of geaggregeerde stadium I tot III melanosomen, of enkele, stadium IV melanosomen gevormd worden. Het zou kunnen dat bij PMH door de hypothetische stof die wordt uitgescheiden door *P. acnes* de regulerende factoren binnen de

keratinocyt worden beïnvloed, wat leidt tot de waargenomen verandering in melanosomen morfologie.

In **Hoofdstuk 6** hebben wij uitgaande van onze bevindingen in vorige onderzoeken een studie uitgevoerd waarbij wij antibacteriële therapie hebben vergeleken met anti-inflammatoire therapie.

In deze studie hebben wij 45 PMH patiënten geïncludeerd. Bij deze patiënten werd de voor- of achterzijde van de romp in twee helften verdeeld. Na randomisatie werd de ene helft behandeld met benzoyl peroxide 5% hydrogel in combinatie met clindamycine 1% lotion eenmaal daags en de andere helft met fluticason crème eenmaal daags. Beide helften ondergingen driemaal per week gedurende 20 minuten UVA fototherapie, om de repigmentatie te bevorderen. Patiënten hebben deze therapieën gedurende 3 maanden voortgezet. Een therapievrije fase van 3 maanden volgde. Van alle patiënten werden foto's gemaakt voor, tijdens en na de behandeling die achteraf beoordeeld werden door drie onafhankelijke dermatologen die geblindeerd waren voor de behandelzijden. Daarnaast werden voor, tijdens en na de behandeling objectieve huidskleurmetingen verricht met de spectrocolorimeter.

Aan het einde van de studie bleek er een significant beter behandelresultaat te zijn aan de zijde die behandeld was met benzoyl peroxide in combinatie met clindamycine dan de zijde die behandeld was met fluticason. Dezelfde resultaten zijn gevonden na een therapievrije follow-up periode van 3 maanden.

Wij hebben geconcludeerd dat de resultaten van deze studie onze hypothese, dat PMH veroorzaakt wordt door *Propioni* bacteriën, ondersteunt.

Hoofdstuk 7 betreft een "letter to the editor". Deze letter is geschreven als reactie op een case report dat gepubliceerd is in the *Journal of the European Academy of Dermatology and Venereology* (Di Lernia en Ricci 2005), waarin verondersteld wordt dat PMH en uitgebreide pityriasis alba (zoals beschreven door Zaynoun *et al*. 1983) dezelfde ziekte zijn. Gezien de overeenkomsten in het klinisch beeld en de electronen microscopie tussen uitgebreide pityriasis alba en PMH is het inderdaad mogelijk dat de twee hetzelfde zijn en dat uitgebreide pityriasis alba niet een ernstige vorm is van pityriasis alba, zoals men in de meeste handboeken veronderstelt.

Conclusie en toekomstperspectieven

De resultaten van de studies in dit proefschrift bevestigen de opvatting dat PMH een aparte, behandelbare, relatief nieuwe entiteit is die indien de juiste diagnostiek wordt toegepast, ook goed te onderscheiden is van andere huidafwijkingen die gepaard gaan met hypopigmentatie. Hoewel de pathogenese nog niet volledig bekend is, hebben wij meer inzicht gekregen in en meer vorm gegeven aan deze ziekte. De aannemelijk gemaakte relatie tussen de *Propioni* bacterie en PMH is een sterk argument in het bevestigen van de opvatting dat het om een apart ziektebeeld gaat. Wij stellen voor om de klinische karakteristieken van PMH in combinatie met rode, folliculaire fluorescentie in de lesionale huid en/of de aanwezigheid van Gram positieve, staafvormige bacteriën in de afvoergangen van de talgklieren van de lesionale huid te beschouwen als de diagnostische criteria voor PMH. Het spreekt voor zich dat nader onderzoek nodig is, waarbij gedacht kan worden aan het consolideren van de diagnostische criteria, het ontwikkelen van nieuwe, aanvullende therapieën en het identificeren van de veronderstelde stof die door *P. acnes* wordt uitgescheiden en die van invloed zou zijn op de melanogenese en/of overdracht van melanosomen.

9 GENERAL DISCUSSION, SUMMARY AND CONCLUSIONS

General discussion, summary and conclusions

In 1987 the first article appeared about a skin disorder that consisted of symmetrically localized, hypopigmented macules on the trunk that according to morphology, course and treatment results, differed from other skin disorders with hypopigmentation (Borelli 1987). In 1988 the name Progressive Macular Hypomelanosis (PMH) was given to this disorder (Guillet *et al*. 1988). In practice this condition was often misdiagnosed as other known skin disorders with hypopigmentation such as pityriasis versicolor.

Over the years more descriptions of similar skin diseases appeared which nowadays we consider to be PMH, however all with different names ("Creole dyschromia" Lesueur *et al*. 1994, "idiopathic multiple large macule hypomelanosis" Fitzpatrick 1996, "nummular and confluent hypomelanosis of the trunk" Menke *et al*. 1997) and systematic research was lacking. In this thesis we systematically studied the pathology, epidemiology, cause, clinical features and treatment of PMH in order to gain evidence that this disorder should be considered a separate entity.

Chapter 1 is an introduction in which we describe the historical background and terminology of PMH. This chapter is a summary of what was known on PMH at the time we started our research.

Chapter 2 provides a description of histological and microbiological findings in PMH. This study was based on clinical finding in patients with PMH of red, follicular fluorescence in lesional skin when examined with a Wood's lamp in a dark room. This fluorescence was absent in the follicles of adjacent normal skin. We suspected the presence of porphyrin producing bacteria, such as *Corynebacterium minutissimum* or *Propionibacterium* species, since red fluorescence is one of the characteristics of these bacteria.

We biopsied skin from follicles of lesional and adjacent (normal) skin and of inter-follicular, lesional and adjacent (normal) skin in 8 PMH patients.

Histological investigation of the follicular, lesional skin showed Gram positive bacteria in the pilosebaceous ducts of all 8 patients. In the follicles of the normal skin and in the inter-follicular skin (lesional as well as normal) these microorganisms were not

observed. Conventional cultivation showed *P. acnes* bacteria in 7 out of 8 biopsies of the follicles of lesional skin. From none of the other biopsies *P. acnes* could be cultured.

We concluded that there seems to be a relation between the presence of *P. acnes* and the hypopigmented macules. Furthermore we speculated that the bacteria might produce a depigmenting substance that interferes with pigment synthesis.

In **Chapter 3** we further investigated the type of *Propioni* bacteria found in PMH. *P. acnes* plays an important role in the cause of acne. However in a previous study (chapter 2) we showed that these bacteria probably also play a role in the pathogenesis of PMH. It is striking that in general most PMH patients do not have acne. This led to the hypothesis that a subtype of *P. acnes* is involved in PMH.
Therefore we conducted a study in which we took biopsies of red follicular fluorescent lesional skin in 14 PMH patients and from a red follicular fluorescent follicle at the site of an acne lesion in 10 acne patients. The biopsies were then smeared on blood agar plates for provisional conventional identification. Next we further analyzed one isolate per plate by the DNA fingerprinting method: amplified fragment length polymorphism (AFLP). For confirmation of our AFLP results we additionally conducted 16S rRNA gene sequencing analysis on isolates from various DNA groups that resulted from the AFLP analysis. These isolates were compared with the reference *P. acnes* strain ATCC 6919. To exclude the presence of a mixture of different species in one patient, we re-sampled 3 acne patients and newly sampled 3 PMH patients. This time a maximum of ten isolates per patient was submitted to DNA fingerprinting by AFLP.

All provisional conventional identified isolates were *P. acnes* bacteria. This was determined by biochemical tests and the accompanying resistance pattern. The AFLP analysis resulted in 3 different DNA groups. Compared to the *P. acnes* reference strain, isolates from group 1 (8 acne and 6 PMH patients) showed a similarity between 55 and 100% suggesting the same species, isolates from group 2 (2 acne patients) showed a similarity between 30 and 55% suggesting a variant of *P. acnes* and group 3 isolates (8 PMH patients) formed a clear distinct DNA group with a similarity of less than 30%. This low level of homology suggested that these isolates belong to a different species. Isolates from group 3 comprised strains isolated solely from PMH patients (n = 8). For these strains a similarity level of < 30% with the reference

P. acnes strain was observed. All clinical strains showed a similarity level of < 30% when compared with *Propionibacterium avidum*, *Propionibacterium granulosum* and *Propionibacterium propionicus*. This suggests another species based on the low level of homology. 16S rRNA gene sequencing analysis of 2 isolates from DNA group 1, 1 isolate from DNA group 2 and 3 isolates from DNA group 3 showed the following: isolates from group 1 showed no difference with the reference strain. The isolates from group 2 showed a single nucleotide difference on position 827 with the reference strain. Two isolates from group 3 showed a single nucleotide difference on position 1243 with the reference strain and 1 isolate from group 3 showed two nucleotide differences on position 712 and 1243. The results of the second AFLP analysis were comparable with our former findings and "mixture" of isolates could be excluded. Additional biochemical tests of bacteria from the different DNA groups confirmed our AFLP findings, however the resistance pattern of the bacteria in the three different groups was exactly the same.

Because of the small differences we found with 16S rRNA gene sequencing analysis and biochemical tests and the comparable resistance pattern between the different DNA groups, we concluded that we are probably dealing with an until now unidentified *Propionibacterium* species. The fact that bacteria from DNA group 3 were only found in PMH patients and not in acne patients, made us conclude that there is a relation between group 3 bacteria and PMH.

Chapter 4 provides a description of PMH within de patient population of the Netherlands Institute for Pigment Disorders.

To gain a better insight in the clinical characteristics and natural course of PMH we conducted a study in which a questionnaire was drawn up that consisted of questions about the skin type of the patient, the beginning of the disorder, the family history, signs and symptoms, the progression of the disorder and previous treatments. Additionally we asked about the medical history, to verify whether relations with other diseases were probable. The questionnaire was sent to 152 PMH patients of whom 101 patients returned a fully completed questionnaire. The results of the questionnaire were then compared with descriptions of the clinical characteristics in the literature to provide a complete overview.

PMH consists of symmetrically localized, hypopigmented, non-scaly macules on the trunk. Usually it starts on the trunk and progresses further on the trunk and in a limited number of patients to the face and the proximal part of the extremities. Often there is confluence of the macules on the trunk. Spontaneous remission probably begins after adolescence, since PMH is practically nonexistent in middle aged people and the elderly. If there is a remission, for instance after treatment, the macules reappear often at exactly the same sites. PMH is mainly seen in adolescents and young adults. The male : female ratio remains indistinct, however most authors observed the disorder especially in women, this corresponds with the distribution in our own patient population. Most of the patients have skin type III to VI and are of mixed (mainly caucasion-negroid) ancestry, although Asians and Mongolians were also mentioned. In most patients a previous diagnosis of pityriasis versicolor was made and patients were often treated with local anti-mycotics. However, in none of the patients these treatments were effective.

Based on the present available information on PMH we concluded that our study gives an as complete as possible description of the clinical characteristics and the course of PMH. Furthermore the results can serve as a guideline for physicians to diagnose PMH in patients with hypopigmentations.

In **Chapter 5** the electron microscopic findings of PMH are described. In this study we searched for ultrastructural correlates of the hypopigmentations to gain more insight into the pathogenesis.

We took 2 mm biopsies from the hypopigmented and normal adjacent skin from 8 PMH patients with skin type III through VI. These biopsies were examined through electron microscopy and the findings were compared.

The results showed that in patients with skin type V and VI the melanosomes that are produced by the melanocytes in the lesional skin of PMH patients can be described as smaller, stage I to III, aggregated melanosomes, while the melanosomes in the normal, adjacent skin can be described as bigger, stage IV, single melanosomes. Stage I to III melanosomes are usually seen in light skin (skin type I through IV). Stage IV melanosomes are seen in colored skin and dark skin (skin type V and VI). The transfer of melanosomes from the melanocyte to the keratinocyte seemed undisturbed. There was no accumulation of melanosomes in the dendrites and the

function of the melanocytes seemed intact, since all precursors of the melanosomes were present in the melanocyte.

We earlier (Chapter 2) proposed the hypothesis that P acnes produces a depigmenting factor. On theoretical grounds two different pathways may lead to the observed change in melanosome formation: a) by inhibiting tyrosinase activity and increasing L-cysteine concentration the hypothetical factor induces a switch from eumelanogenesis to pheomelanogenesis, resulting in smaller, aggregated melanosomes (stage I to III) and thus a lighter skin; so in this proposal the target of the hypothetical factor is the melanocyte; b) recent studies showed that factors within the keratinocytes determine whether aggregated, stage I to III melanosomes, or single, stage IV melanosomes are produced. The hypothetical factor produced by P acnes might affect the regulatory factors within the keratinocyte, leading to the production of smaller aggregated melanosomes.

In **Chapter 6** we conducted a study based on previous findings, in which we compared antibacterial therapy with anti-inflammatory therapy.

In this study we included 45 PMH patients. In these patients the front or back side of the trunk was divided in two halves. After randomization one side was treated with benzoyl peroxide 5% hydrogel in combination with clindamycin 1% lotion once daily and the other side was treated with fluticasone cream once daily. Both sides were treated with UVA phototherapy three times a week during 20 minutes. Patients were treated with these therapies for three months. Pictures were taken of all patients before, during and after treatment. The pictures were assessed by three independent dermatologists who were blinded for treatment sides. Furthermore before, during and after treatment objective skin color measurements were performed with a spectrocolorimeter.

At the end of the study a significant better treatment result was observed on the side that was treated with benzoyl peroxide in combination with clindamycin than the side treated with fluticasone. The same results were found after a therapy-free follow up period of 3 months.

We concluded that the results of this study support our hypothesis that PMH is caused by a *Propionibacterium*.

Chapter 7 concerns a "letter to the editor". This letter was written as a reaction to a case report published in the *Journal of the European Academy of Dermatology and Venereology*, in which the authors (Di Lernia and Ricci 2005) implied that PMH and extensive pityriasis alba (as described by Zaynoun *et al*. 1983) are the same disease. Because of the clinical and ultrastructural similarities between extensive pityriasis and PMH we believe they are similar and that extensive pityriasis alba is not a severe form of pityriasis alba, like most handbooks imply.

Conclusion and future perspectives

The results of the studies presented in this thesis confirm the opinion that PMH is a separate, treatable and relatively new entity, which if the right diagnostic tests are performed can easily be distinguished from other hypopigmented skin disorders. Although the precise pathogenesis is still unknown, we gained more insight and gave a more accurate description of PMH. The investigations demonstrating the relation between *Propioni* bacteria and PMH provide the strongest evidence in confirming the idea that it is a separate entity. We propose that the clinical characteristics of PMH in combination with red, follicular fluorescence in lesional skin and/or gram-positive bacteria with a rod-like structure in the pilosebaceous glands of lesional skin, should be considered as diagnostic criteria for PMH. It is obvious that further research is necessary, for instance to consolidate the diagnostic criteria, to develop additional treatment modalities and to identify the postulated factor that is produced by the bacteria, influencing melanogenesis.

10

REFERENCES

BIBLIOGRAPHY

DANKWOORD

COLOR FIGURES

References

Alaluf S, Atkins D, Barrett K, Blount M, Carter N and Heath A. The impact of epidermal melanin on objective measurements of human skin color. *Pigment Cell Res* 2002;15:119–126.

Blessmann Weber M, Sponchiado de Avila LG, Albaneze R, Magalhaes de Oliveira OL, Sudhaus BD, Cestari TF. Pityriasis alba: a study of pathogenic factors. *J Eur Acad Dermatol Venereol*. 2002;16(5):463-468.

Bommer S. Hautuntersuchungen im gefilterten Quartzlicht. *Klin Wochenschr.* 1927;6:1142-1144.

Borelli D. Cutis trunci variata: nueva genodermatosis. *Med Cutan Ibero-Lat-Am.*1987;15:317-319.

Breathnach AS, Nazzaro-Porro M, Martin B. Ultrastructure of skin in pityriasis versicolor. *G Ital Dermatol.* 1975;110:457-469.

Britton WJ, Lockwood DN. Leprosy. *Lancet*. 2004;363(9416):1209-1219.

Burkhart CN. Digital fluorescence as a parameter of *Propionibacterium acnes* suppression needs assessment. *Int J Dermatol.* 2001;40:101-103.

Charles CR, Sire DJ, Johnson BL, Beidler JG. Hypopigmentation in tinea versicolor: a histochemical and electronmicroscopic study. *Int J Dermatol.* 1973;12:48-58.

Clarridge JE 3rd. Impact of 16S rRNA gene sequence analysis for identification of bacteria on clinical microbiology and infectious diseases. *Clin Microbiol Rev*. 2004;17(4):840-862.

Crespo EV, Delgado FV. Malassezia species in skin diseases. *Curr Opin Infect Dis*. 2002;15:133-142.

Dasen G, Smutny J, Teuber M, Meile L. Classification and identification of Propionibacteria based on ribosomal RNA genes and PCR. *Syst Appl Microbiol*. 1998;21(2):251-259.

Del Marmol V, Ito S, Bouchard B et al . Cysteine deprivation promotes eumelanogenesis in human melanoma cells. J Invest Dermatol 1996;107:698-702.

Di Lernia V, Ricci C. Progressive and extensive hypomelanosis and extensive pityriasis alba: same disease, different names? *JEADV*. 2005;19:370-372.

Ellis CN, Leyden JJ, Katz HI, Goldfarb MT, Hickman J and Jones TM *et al.* Therapeutic studies with a new combination benzoyl peroxide/clindamycin topical gel in acne vulgaris. *Cutis* 2001;67:13-20.

Fitzpatrick TB in Sober AJ, Fitzpatrick TB, editors. Yearbook of Dermatology. St Louis, Mo: Mosby-Year Book; 1996:416-417.

Galadari I, El Komy M. Tinwa versicolor: histologic and ultrastructural investigation of pigmentary changes. *Int J Dermatol.* 1992;31:253-256.

Guillet G, Gauthier Y, Helenon R. Hypomélanose maculaire progressive du tronc (hypopigmentation primitive acquise des Antilles) *Soc Fr Dermatol.* (Paris), mars 1985.

Guillet G, Helenon R, Gauthier Y, Surleve-Bazeille JE, Plantin P, Sassolas B. Progressive macular hypomelanosis of the trunk: primary acquired hypopigmentation. *J Cutan Pathol*. 1988;15:286-289.

Guillet G, Helenon R, Guillet MH, Gauthier Y, Menard N. Hypomelanose maculeuse confluente et progressive du metis melanoderme. *Ann Dermatol Venereol*. 1992;119(1):19-24.

Hay J, Moore MK. Mycology. In: Burns T, Breathnack S, Cox N, Griffiths C (Eds). Rook's Textbook of Dermatology. Volume II. 7th edition. Massachusetts: Blackwell Science; 2004. p. 31.1-31.14.

Holden CA, Berth-Jones J. Eczema, Lichenification, Prurigo and Erythroderma. In: Burns T, Breathnack S, Cox N, Griffiths C (Eds). Rook's Textbook of Dermatology. Volume I. (7th ed.). Massachusetts: Blackwell Science; 2004. p. 17.37-17.38.

Hurwitz S. Eczematous eruptions in childhood. In: Paller AS, Mancini AJ, (Eds). Clinical Pediatric Dermatology: A Textbook of Skin Disorders of Childhood and Adolescence, (3rd ed.). Philadelphia: WB Saunders; 2006. p.49.

Jimbow K, Sugiyama S. Melanosomal translocation and transfer. In: Nordlund JJ, Boissy RE, Hearing VJ, King RA, Ortonne J-P (eds.). The Pigmentary System. Oxford University Press, Oxford, 1998. p. 107-114.

Johnson, J. L. and C. S. Cummins. Cell wall composition and deoxyribonucleic acid similarities among the anaerobic coryneforms, classical *Propionibacteria*, and strains of Arachniapropionica. *J. Bacteriol.* 1972;109:1047-1066.

Johnsson A, Kjeldstad B, Melø TB. Fluorescence from pilosebaceous follicles. *Arch Dermatol Res.*1987;279(3):190-193.

Kumarasinghe SP, Tan SH, Thng S, Thamboo TP, Liang S, Lee YS. Progressive macular hypomelanosis in Singapore: a clinico-pathological study. *Int J Dermatol*. 2006;45(6):737-742.

Lambroza E, Cohen SR, Phelps R, Lebwohl M, Braverman IM, DiCostanzo D. Hypopigmented variant of mycosis fungoides: demography, histopathology, and treatment of seven cases. *J Am Acad Dermatol*. 1995;32(6):987-993.

Lesueur A, Garcia-Granel V, Helenon R, Cales-Quist D. Hypomélanose maculeuse confluente et progressive du métis mélanoderme: etude epidemiologique sur 511 subjets. *Ann Dermatol Venereol.*1994;121:880-883.

Leyden JJ, McGinley KJ, Mills OM, Kligman AM. Age-related changes in the resident bacterial flora of the human face. *J Invest Dermatol.* 1975;65:379-381. Leyden JJ, Hickman JG, Jarratt MT, Stewart DM and Levy SF. The efficacy and safety of a combination benzoyl peroxide/clindamycin topical gel compared with benzoyl peroxide alone and a benzoyl peroxide/erythromycin combination product. *J Cutan Med Surg*. 2001;5:37-42.

Leyden JJ. A review of the use of combination therapies for the treatment of acne vulgaris. *J Am Acad Dermatol.* 2003;49(Suppl):S200-210.

Lin RL, Janniger CK. Pityriasis alba. *Cutis*. 2005;76(1):21-24.

McDowell A, Valanne S, Ramage G, Tunney MM, Glenn JV, McLorinan GC *et al*. *J. Clin. Microbiol*. 2005;43(1):326-334.

McDowell A, Perry AL, Lambert PA, Patrick S. A new phylogenetic group of Propionibacterium acnes. *J Med Microbiol*. 2008 Feb;57(Pt 2):218-224.

McGinley KJ, Webster GF, Leyden JJ. Regional variations of cutaneous propionibacteria. *Appl Environ Microbiol.* 1978;35:62-66.

McGinley KJ, Webster GF, Leyden JL. Facial follicular porphyrin fluorescence: correlation with age and density of *Propionibacterium acnes. Br J Dermatol.* 1980;102:437-441.

Menke HE, Doornweerd S, Zaal J, Roggeveen C, Dingemans KP, van der Bergh Weerman M, Westerhof W. Acquired nummular and confluent hypomelanosis of the trunk. Paper presented at: Second annual meeting of the European Society for Pigment Cell Research: June 18-21 1989; Uppsala, Sweden.

Menke HE, Ossekoppele R, Dekker SK, v Praag MCG, Zaal J, Noordhoek-Hegt V, Westerhof W. Nummulaire en confluerende hypomelanosis van de romp. *Ned Tijdsch Dermatol Venereol*. 1997;7:117-122.

Menke HE, Njoo D, Westerhof W. Progressive hypomelanosis of the trunk. In: Nordlund JJ, Boissy RE, Hearing VJ, King RA, Ortonne J-P (eds.). *The Pigmentary System.* New York, NY: Oxford University Press; 1998. p. 686-688.

Menke HE, Relyveld G, Njoo D, Westerhof W. Progressive macular hypomelanosis. In: JJ Nordlund *et al.* (eds.). The pigmentary system. (2nd ed.). Malden Massachusetts: Blackwell Publishing; 2006. p.748-751.

Minwalla L, Zhao Y, Le Poole IC, Wickett RR, Boissy RE. Keratinocytes play a role in regulating distribution patterns of recipient melanosomes in vitro. *J Invest Dermatol.* 2001;117:341-347.

Mohammadi T, Reesink HW, Pietersz RN, Vandenbroucke-Grauls CM, Savelkoul PH. Amplified-fragment length polymorphism analysis of Propionibacterium isolates implicated in contamination of blood products. *Br J Haematol*. 2005;131(3):403-409.

Morishita N, Sei Y. Microreview of Pityriasis versicolor and Malassezia species. *Mycopathologia*. 2006;162(6):373-376.

Naafs B and Faber WR. Leprosy in Faber WR, Hay RJ and Naafs B (edit) Imported skin diseases. Elsevier 2006 Maarssen, 1st ed. p. 89-101.

Nazzaro-Porro M, Passi S, Picardo M, Mercantini R, Breathnach AS. Lipoxygenase activity of pityrosporum in vitro and in vivo. *J Invest Dermatol.* 1986;87:108-112.

Nickoloff BJ. Light-microscopic assessment of 100 patients with patch/plaque-stage Mycosis fungoides.. *Am J Dermatopathol*. 1988;10(6):469-477. Review

Ortonne JP, Bhadoran P, Fitzpatrick TB, Mosher DB and Hori Y. Progressive macular hypomelanosis of the trunk. In: I. Freedberg, A. Eisen, K. Wolff, F. Austen, L. Goldsmith and S. Katz (eds.). *Fitzpatrick's dermatology in general medicine* (6th ed.), McGraw-Hill 2003 p. 861-862.

Pardillo FE, Fajardo TT, Abalos RM, Scollard D, Gelber RH. Methods for the classification of leprosy for treatment purposes. *Clin Infect Dis*. 2007;44(8):1096-1099. Epub 2007 Mar 5.

Prohic A, Ozegovic L. Malassezia species isolated from lesional and non-lesional skin in patients with pityriasis versicolor. *Mycosis*. 2007;50(1):58-63.

Relyveld GN, Kingswijk MM, Reitsma JB, Menke HE, Bos JD, Westerhof W. Benzoyl peroxide/clindamycin/UVA is more effective than fluticasone/UVA in progressive macular hypomelanosis: a randomized study. *J Am Acad Dermatol*. 2006;55(5):836-843.

Relyveld G, Menke H, Westerhof W. Progressive and extensive hypomelanosis and extensive pityriasis alba: same disease, different names? *J Eur Acad Dermatol Venereol.* 2006;20:1363-1364.

Relyveld GN, Menke HE, Westerhof W. Progressive macular hypomelanosis: an overview. *Am J Clin Dermatol.* 2007;8(1):13-19.

Relyveld GN, Dingemans KP, Menke HE, Bos JD, Westerhof W. Ultrastructural findings in progressive macular hypomelanosis indicate decreased melanin production. *J Eur Acad Dermatol Venereol*. 2008;22(5):568-574.

Ridley DS, Jopling WH. Classification of leprosy according to immunity. A five group system. *Int J Lepr* .1966. p. 34-37.

RIVM, Rijksinstituut voor Volksgezondheid en Milieu: www.rivm.nl. Nationaal Kompas Volksgezondheid, versie 3.13, april 2008.

Roberts SOB. Pityriasis versicolor: a clinical and mycological investigation. *Br J Dermatol*.1969; 81:315-326.

Savelkoul PH, Aarts HJ, de Haas J, Dijkshoorn L, Duim B, Otsen M, Rademaker JL, Schouls L, Lenstra JA. Amplified-fragment length polymorphism analysis: the state of an art. *J Clin Microbiol*. 1999;37(10):3083-3091.

Shapiro PE, Pinto FJ.The histologic spectrum of mycosis fungoides/Sézary syndrome (cutaneous T-cell lymphoma). A review of 222 biopsies, including newly described patterns and the earliest pathologic changes. *Am J Surg Pathol*. 1994;18(7):645-667.

Shriver MD and Parra EJ. Comparison of narrow-band reflectance spectroscopy and tristimulus colorimetry for measurements of skin and hair color in persons of different biological ancestry. *Am J Phys Anthropol.* 2000;112:17-27.

Smith NP, Samman PD. Mycosis fungoides presenting with areas of cutaneous hypopigmentation. *Clin Exp Dermatol* . 1978;3:213-216.

Smit NP, Van der Meulen H, Koerten HK et al . Melanogenesis in cultured melanocytes can be substantially influenced by 1-tyrosine and 1-cysteine. J Invest Dermatol 1997;109:796-800.

Sober AJ, FitzPatrick TB. *Yearbook of Dermatology.* St Louis, Mo: Mosby–Year Book; 1996:416-417.

Szabo G. Racial differences in the fate of melanosomes in human epidermis. *Nature.* 1969;222:1081-1082.

Tancrede, C. Role of human microbiota in health and disease. *Eur J Clin Microbiol Infect Dis.* 1992;11:1012-1015.

Toda K, Pathak MA, Parrish JA, Fitzpatrick TB, Quevedo WC Jr. Alteration of racial differences in melanosome distribution in human epidermis after exposure to utraviolet light. Nat New Biol 1972;236:143-145.

Toyoda M, Morohashi M. Pathogenesis of acne. *Med Electron Microsc.* 2001;34:29-40.

Vargos-Ocampo F. Pityriasis alba: a histologic study. *Int J Dermatol*. 1993;32:870-873.

Urano-Suehisa S, Tagami H. Functional and morphological analysis of the horny layer of pityriasis alba. *Acta Derm Venereol.* 1985;65:164-167.

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, et al. AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.*1995;23(21):4407-4414.

Wagner JK, Parra EJ, Norton L, Jovel C and Shriver MD. Skin responses to ultraviolet radiation: effects of constitutive pigmentation, sex, and ancestry, *Pigment Cell Res.* 2002;15:385-390.

Weedon D, ed. Pityriasis alba. In: *Skin Pathology*, 2nd edn. Churchill Livingstone Elsevier Science Limited, London, 2002. p. 327.

Westerhof W, Relyveld GN, Kingswijk MM, de Man P, Menke HE. Propionibacterium acnes and the pathogenesis of progressive macular hypomelanosis. *Arch Dermatol*. 2004;140(2):210-214.

Whittaker SJ, MacKie RM. Cutaneous lymphomas and lymphocytic infiltrates. In: Burns T, Breathnack S, Cox N, Griffiths C (Eds). Rook's Textbook of Dermatology. Volume III. (7th ed.). Massachusetts: Blackwell Science; 2004. p. 54.2-54.13.

Wolff K, Konrad K. Phagocytosis of latex beads by epidermal keratinocytes in vivo. *J Ultrastruct Res.* 1972;39:262-280.

Wolff K, Jimbow K, Fitzpatrick TB. Experimental pigment donation in vivo. J Ultrastruct Res 1974;47:400-419.

Yamamoto O, Bhawan J. Three modes of melanosome transfers in Caucasion facial skin: hypothesis based on an ultrastructural study. *Pigment Cell Res/* 1994;7:58-169.

Yawalkar SJ. Leprosy for medical practioners and paramedical workers. (7th ed.). 2002 Novartis foundation for sustainable development, Basle Switzerland. p. 17-33.

Zaynoun ST, Aftimos BG, Tenekjian KK, Bahuth N, Kurban AK. Extensive pityriasis alba: a histological histochemical and ultrastructural study. *Br J Dermatol*. 1983;108(1):83-90.

Zaynoun ST, Abi Ali Jaber L, Kurban AK. Oral methoxsalen photochemotherapy of extensive pityriasis alba. *J Am Acad Dermatol*.1986;15:61-65.

Bibliography

Menke H, Relyveld G, Westerhof W. Comment on the letter by Chung et al. about progressive macular hypomelanosis. *J Eur Acad Dermatol Venereol*. 2008 Jun 5. (Epub ahead of print).

Relyveld GN, Dingemans KP, Menke HE, Bos JD, Westerhof W. Ultrastructural findings in progressive macular hypomelanosis indicate decreased melanin production. *J Eur Acad Dermatol Venereol*. 2008 May;22(5):568-574.

Appelman A, Relyveld GN, van Gool T, de Vries HJC. Uw diagnose? *Tijdschr Infect*. 2007;2:153 en 157.

Relyveld GN, Menke HE, Westerhof W. Progressive macular hypomelanosis: an overview. *Am J Clin Dermatol*. 2007;8(1):13-19.

Relyveld GN, Kingswijk MM, Reitsma JB, Menke HE, Bos JD, Westerhof W. Benzoyl peroxide/clindamycin/UVA is more effective than fluticasone/UVA in progressive macular hypomelanosis: a randomized study. *J Am Acad Dermatol*. 2006 Nov;55(5):836-843.

Menke HE, Relyveld G, Njoo D, Westerhof W. Progressive macular hypomelanosis. In: JJ Nordlund et al (eds). *The pigmentary system*. 2nd ed. Malden Massachusetts: Blackwell Publishing; 2006 p.748-751.

Relyveld G, Menke H, Westerhof W. Progressive and extensive pityriasis alba: same disease, different names? *J Eur Acad Dermatol Venereol.* 2006;20(10):1363-1364.

Menke HE, Relyveld GN, Westerhof W. Progressieve maculaire hypomelanose. *Ned Tijdschr Dermatol Venereol.* 2005;3:123-125.

Westerhof W, Relyveld GN, Kingswijk MM, de Man P, Menke HE. *Propionibacterium acnes* and the pathogenesis of progressive macular hypomelanosis. *Arch Dermatol*. 2004;140(2):210-214.

Westerhof W, Relyveld GN, Kingswijk MM, Menke HE. Wordt progressieve maculaire hypomelanose veroorzaakt door *Propionibacterium acnes*? *Ned Tijdschr Dermatol Venereol*. 2004;14(8):314-317.

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Mitra mijn paranimf, we hebben in de korte tijd dat wij elkaar kennen samen veel meegemaakt. Jou integriteit, kracht en inzet zijn bewonderenswaardig. Ik had geen betere paranimf kunnen kiezen. Ik wens je heel veel succes toe met de rest van je carrière. Je bent een top collega.

En last, but certainly not least, Gideon, mijn lieve man en paranimf, jij bent mijn steun en toeverlaat geweest. Als het even niet mee zat, wist jij altijd weer een manier te bedenken om mij op te vrolijken. Jij hebt mij leren relativeren en doen inzien dat stress nergens voor nodig is. We hebben ondanks onze drukke werkschema's altijd een manier gevonden om samen te genieten van de goede dingen in het leven en fantastische reizen kunnen maken. Ik heb er alle vertrouwen in dat jou promotie ook snel zal volgen. Voor mij ben jij echt "the king":). Liefs,

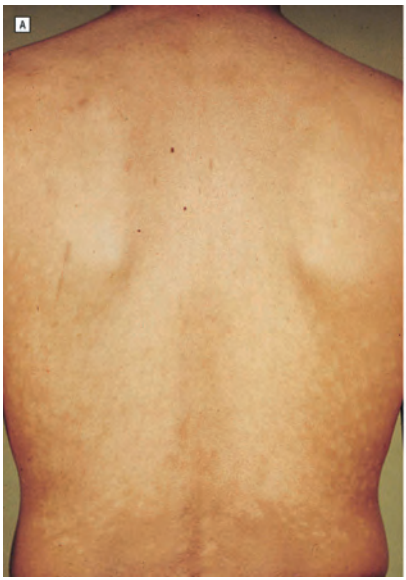


Figure 2-I A, B

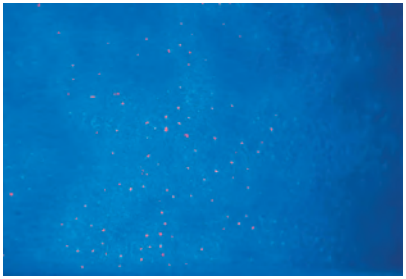
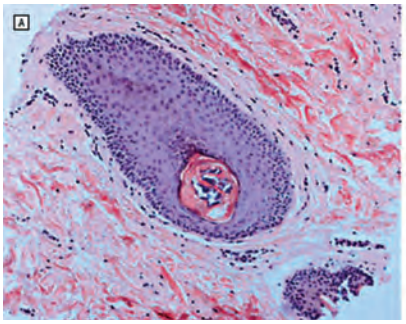


Figure 2-2



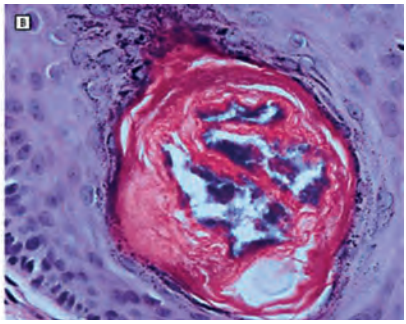


Figure 2-3 A, B

Chapter 3

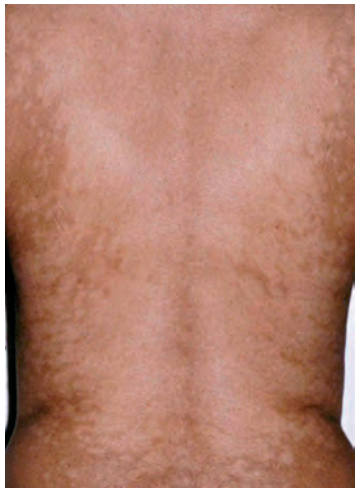


Figure 3-I

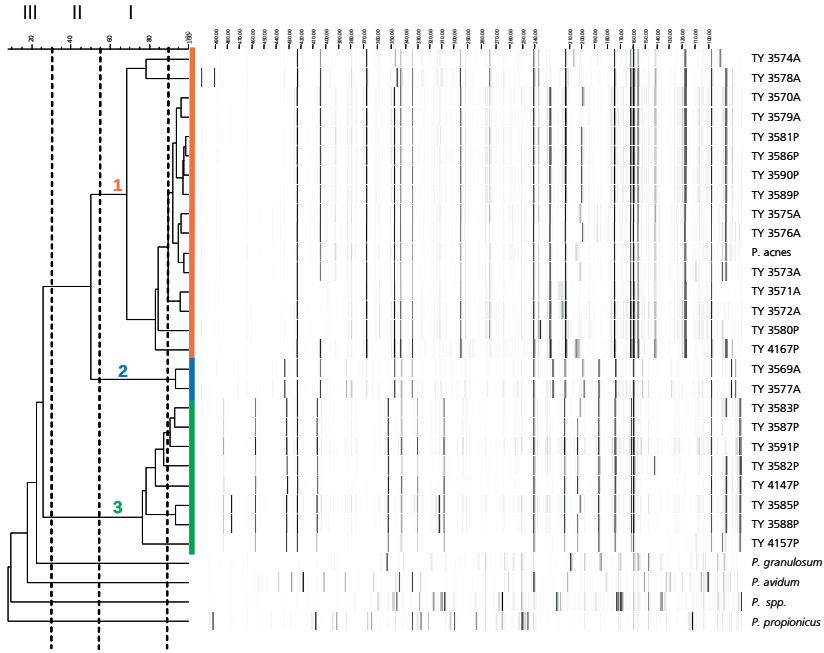
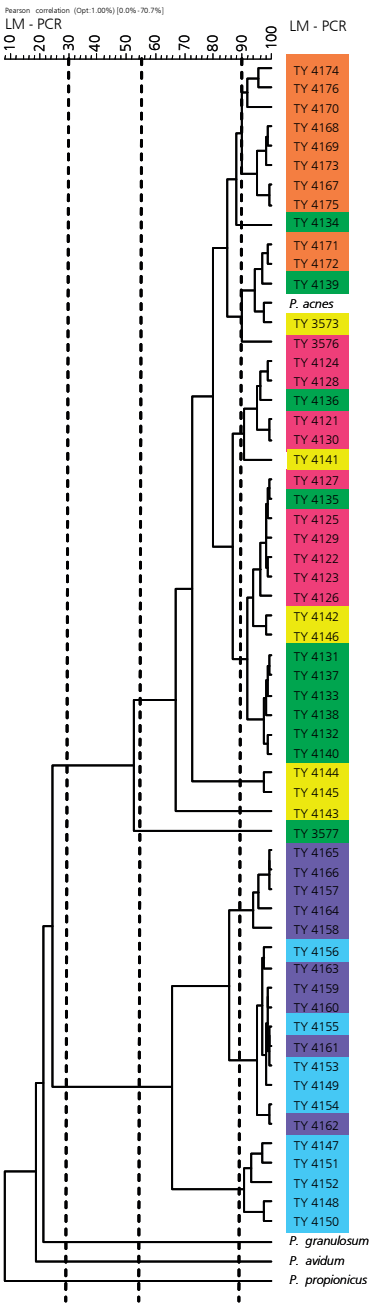


Figure 3-2


Pearson correlation (Opt:1.00%) [0.0% - 70.7%]
LM - PCR
10
20
30
40
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60
70
80
90
100
LM - PCR
TY 4174
TY 4176
TY 4170
TY 4168
TY 4169
TY 4173
TY 4167
TY 4175
TY 4134
TY 4171
TY 4172
TY 4139
P. acnes
TY 3573
TY 3576
TY 4124
TY 4128
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TY 4154
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TY 4148
TY 4150
P. granulosum
P. avidum
P. propionicus

Figure 3-3

Chapter 4

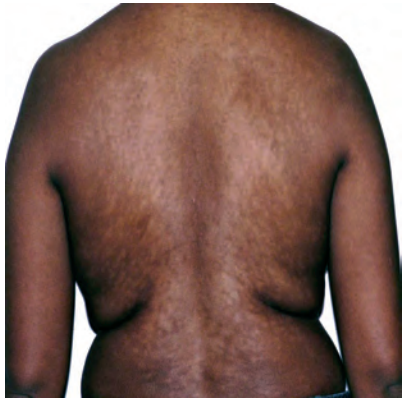


Figure 4-1

Chapter 5

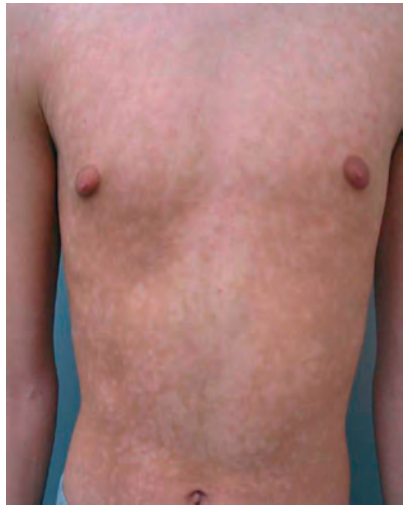
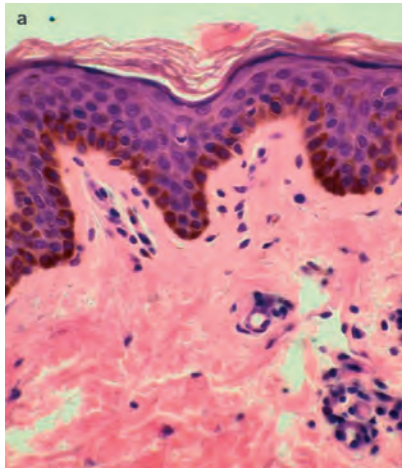


Figure 5-1



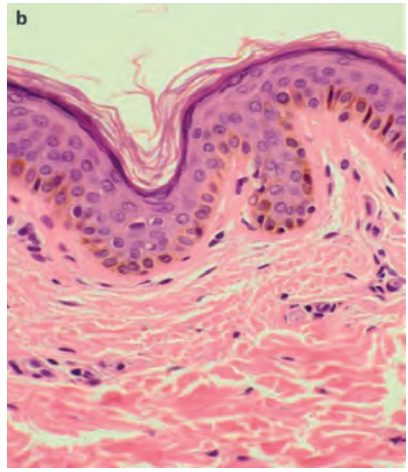


Figure 5-2

Chapter 6

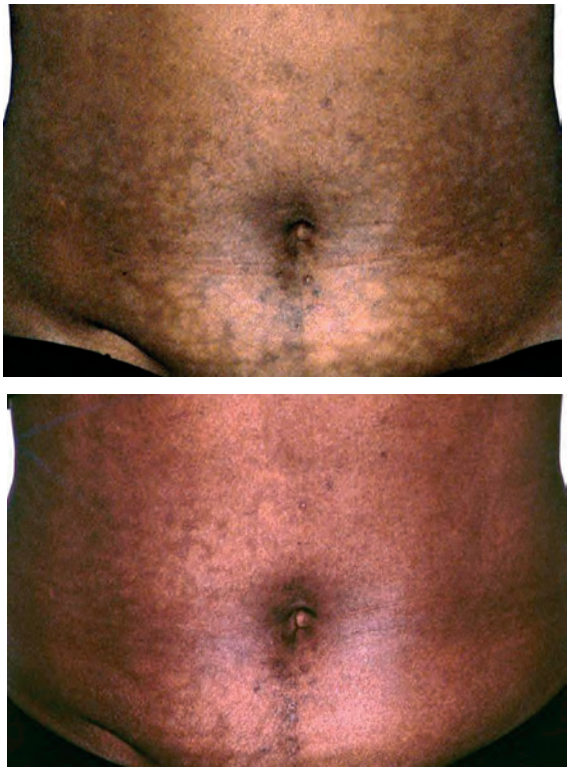


Figure 6-4 A,B