Title:

Is *Cutibacterium* (previously *Propionibacterium*) *acnes* a potential pathogenic factor in the aetiology of the skin disease progressive macular hypomelanosis?

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Abstract

Progressive macular hypomelanosis (PMH) is a skin condition that normally causes symmetrically distributed hypopigmented macules on the front and back of the trunk, but rarely the face. To date, the pathophysiology of the condition is not well understood, but a role for the anaerobic skin bacterium *Cutibacterium* (previously *Propionibacterium*) *acnes* in the development of the disease has been proposed due to its sole presence within lesional, but not normal peri-lesional, skin. The success of antimicrobials in the treatment of PMH also provides circumstantial evidence that this association may be causal, although this is still to be proven. More recent culture and metagenomic typing studies indicate that strains of *C. acnes* subsp. *elongatum* (type III) may be important in the aetiology of the condition, which would help to explain why PMH does not normally affect the face since such strains are rarely present there, and why no association between this condition and acne vulgaris is found; acne appears to primarily involve type IA_1 strains from *C. acnes* subsp. *acnes* (type I). In this review we summarise current knowledge on the relationship between *C. acnes* and PMH, and re-examine previous challenges to the view that the bacterium plays a role in the condition against the backdrop of newly emerged data.

1. Introduction

Progressive macular hypomelanosis (PMH) is a relatively uncommon dermatosis usually characterised by ill-defined nummular, non-scaly and symmetric hypopigmented macules that form predominantly on the front and back of the trunk, and without prior history of skin injury, infection or inflammation (Fig. 1); these macules can be discrete or display confluence when found in and around the midline[1]. Like the very common skin condition acne vulgaris, PMH normally affects adolescents and young adults where its cosmetic effects may have social and psychological impacts[1,2,3]. The disorder rarely affects the proximal extremities and the skin of the face, which has been a key clinical feature[1,3]. PMH is found worldwide and affects different Fitzpatrick skin types as well as both sexes, but the disorder does appear to be much more prevalent in females[1,4-7]. PMH is often misdiagnosed as the fungal infection pityriasis versicolor, pityriasis alba or post-inflammatory hypopigmentation, and can remain stable, progress slowly over a long time period or, in some cases, spontaneously disappear after mid-life[1,3,4,8]. While the exact incidence of PMH within different populations is unclear, it is likely to be an under-reported disorder as some cases may be misdiagnosed and not all patients, particularly males, will see a clinician for treatment[6]. In this review, we evaluate the strength of current evidence supporting a pathogenic role for the anaerobic bacterium *Cutibacterium acnes* (previously *Propionibacterium acnes*) in the aetiology of PMH, particularly in light of recent culture and metagenomic typing studies, and investigation of porphyrin production by different strains of the bacterium.

2. Histological and electron microscopic features

Histologically, PMH does not appear to be associated with any significant abnormalities of the dermis apart from a decrease in epidermal pigment, but a mild perifollicular infiltrate of lymphocytes has been observed in some, but by no means all, lesional skin samples[2,4,6-10].

Detailed ultrastructural studies have provided evidence that PMH results from altered melanogenesis, leading to reduced pigmentation, and changes in melanosome size, aggregation, maturation and distribution[2,3,6,8-9,11-12]. Furthermore, it does not appear that defects in melanosome degradation play a role in the pathophysiology of PMH as there is no evidence for disintegrated melanosomes in the lysosomal compartments of PMH lesions[12].

3. Evidence supporting a potential role for *Cutibacterium acnes* in the aetiology of PMH

While the underlying cause of PMH is still not clear, a pathogenic role for the Gram-positive, anaerobic bacterium *C. acnes* in the aetiology of the condition has been proposed, possibly via the production of a depigmenting factor or an agent that interferes with melanogenesis[4,5]. *C. acnes* is part of the normal human microbiota and found predominately on the skin and muscosal surfaces. The organism is an opportunistic pathogen most noted for its association with acne vulgaris[13], but has now also been linked to other human infections and conditions, including medical device and soft tissue infections[14,15], cervical disc disease[16], prostate cancer[17] and sarcoidosis[18].

3.1 Culture and non-culture-based detection

Bacterial culture has demonstrated the sole presence and accumulation of *C. acnes* in biopsy samples of PMH lesions, but not biopsies of adjacent non-lesional skin from the same patient[4,5] (Table 1). Furthermore, 16S rRNA-based quantitative real-time PCR detection of *C. acnes* has found a significantly greater incidence of the bacterium in lesional versus adjacent non-lesion skin of patients in respect to genome copy number, consistent with culture results and indicating enrichment in lesions[5]; in the latter case we can speculate that this may reflect localised perturbations in the skin environment that stimulate overgrowth. The presence of *C. acnes* within hypopigmented lesions from patients with PMH, but not normal pigmented skin from the trunk of the same patient, can also be observed upon Gram-staining, revealing

Gram-positive rods with a high population density[1,4,9]. Furthermore, upon examination of the skin in a dark room with UV radiation from a Wood's lamp, a puntiform orange-red follicular fluorescence within hypopigmented spots is observed due to the presence of porphyrins produced by the bacterium, such as coproporhyrin III; this fluorescence is absent in peri-lesional normal skin[1,4,7,10,12]. Interestingly, while this characteristic florescence of PMH lesions upon Wood's lamp examination has been described in many studies, it has not been observed in all (see section 5.1).

3.2 Therapeutic success of antimicrobial-based treatments

Treatments for PMH based on topical corticosteroids and topical or systemic antifungals have not proved efficacious, but re-pigmentation of the skin can be achieved using ultraviolet light A (UVA) or narrow-band UVB (NB-UVB)-based treatments, either as a monotherapy or in combination with topical or oral antimicrobials[1,6,9,10,19-24]. Such UV treatments are believed to work by stimulating melanogenesis and, potentially, inhibition of *C. acnes* in the case of NB-UVB (see section 5.3), but the results appear variable and in many cases are only temporary leading to recurrence of the condition[6,20,21,24.]

In a within-patient, left-right trunk comparison study, Relyeld et al.[25] demonstrated that topical treatment with 5% benzoyl peroxide (BPO) (morning) and 1% clindamycin hydrogel (night-time) in combination with UVA radiation (antibacterial therapy arm) was much superior for re-pigmentation versus 0.05% fluticasone and UVA treatment only (anti-inflammatory therapy arm). This appeared, therefore, to exclude the possibility that treatment success was due solely to UVA treatment, and provided evidence to support a bacterial role, such as *C. acnes*, in the pathophysiology of PMH. Furthermore, upon a 3-month follow-up, PMH patients were still found to have retained their re-pigmentation, although information on whether this persisted is not available. Since then, there have been many

studies investigating the effectiveness of treatments for PMH using topical antibiotic lotions and BPO hydrogels (in combination or separately) alongside narrow-band UVB (NB-UVB) treatment[6,9,19,21,23]. Treatment success for PMH has also been achieved using the oral tetracycline derivatives doxycycline, minocycline and lymecycline, used in the management of acne, either with or without BPO[26-29]; this provides further circumstantial evidence that *C. acnes* may contribute to the development of PMH. In particular, treatment of PMH with a combination of oral lymecycline (300 mg/d) and topical 5% BPO was very successful leading to repigmentation and maintenance during a 6-to-12 month follow-up period[28,29]. Attempts to treat PMH using oral isotretinoin have also been described in the literature, but the results obtained have been variable[30-31].

4. The hunt for a novel *Cutibacterium* species associated with PMH

A conundrum in the proposal that *C. acnes* is the cause of PMH has been why the disorder, unlike acne, rarely affects the face where levels of the bacterium are at their highest, and why acne does not predispose individuals to PMH development. This led Relyveld et al.[32] to propose that the organism potentially causing PMH may not actually be *C. acnes*, but a closely related *Cutibacterium* species indistinguishable by conventional phenotypic/ biochemical methods.

4.1 Amplified Fragment Length Polymorphism and 16SrRNA gene analysis

Genetic analysis of skin biopsy-associated bacterial isolates collected from patients with PMH and patients with acne by Amplified Fragment Length Polymorphism (AFLP) typing identified three major genetic clusters that differed in their distribution between the two conditions ($p<0.01$; Freeman-Halton extension of Fisher's extact test) (Table 2)[32]. Of note was the observation that isolates from DNA group 3, in contrast to the other DNA groups, were only associated with PMH, but never acne (Fishers exact test; $p<0.01$), and analysis of

multiple bacterial colonies isolated from acne and PMH samples did not demonstrate the presence of mixed AFLP types. 16S rRNA gene sequencing revealed very high sequence identities between all clusters, with only a single nucleotide polymorphism (SNP) at position 827 separating DNA groups I and II[32], which is a characteristic difference between the well described *C. acnes* type I (*C. acnes* subsp. *acnes*) and type II (*C. acnes* subsp. *defendens*) phylotypes[33], while group 3 isolates differed from group 1 due to a SNP at position 1243 (G1243A). While biochemical analysis with the rapid ID 32A multi-test identification system confirmed DNA groups 1 and 2 as *C. acnes* (99.9% certainty), isolates from DNA group 3 gave ambiguous results and could not be identified phenotypically despite the molecular results indicating a unique *C. acnes* cluster; 16S rRNA identity is not, however, always a guarantee of species identity, especially in the case of a recently diverged and very closely related sister taxon[34]. This led to the proposal that organisms from DNA group 3 may represent a novel and very closely related bacterium from the genus *Cutibacterium*[32].

4.2 PCR phylotyping and single- and multi-locus sequence type analysis

At the time of the original AFLP study of Relyveld et al.[32], knowledge on the intraspecies diversity of *C. acnes* was only developing, as were the molecular methods for more detailed population genetic analysis of the bacterium. Today, our appreciation of *C. acnes* at the interspecies level is much more complete (Table 3), and specific molecular typing tools for the bacterium, particularly multiplex-PCR phylotyping, high-resolution single and multilocus sequence typing (HR-SLST and MLST, respectively) and ribotyping[35-39], have also been established enabling researchers to deeper explore the association of specific lineages with skin health and disease.

Against this new landscape of understanding, and utilising the improved typing methods now available, Barnard et al.[40] conducted a population genetic analysis of *C. acnes* isolates

158 recovered from the lesional skin of patients with PMH. They demonstrated a strong statistical
159 association between strains from the more recently described type III phylogenetic lineage
160 (now known as *C. acnes* subsp. *elongatum*[41,42]) and lesions, but not those representing other
161 phylogenetic groups, including those associated with acne (type IA_1). Strikingly, *in silico* 16S
162 rDNA SNP analysis revealed that the isolates from AFLP DNA group 3 (G1243A) previously
163 described in association with PMH were also consistent with the type III lineage (Fig. 2).
164 Furthermore, analysis of the biochemical phenotype of three representative type III strains
165 from PMH lesions using the Rapid ID 32A multi-tests identification system failed to correctly
166 identify the isolates as *C. acnes,* consistent with the previous results obtained for AFLP DNA
167 group 3 strains[32].
168 A subsequent study by Peterson et al.[29] based on HR-SLST metagenomic analysis of skin
169 surface swabs taken from 24 PMH back lesions and adjacent non-lesional skin regions of eight
170 female patients, confirmed the association of type III strains with PMH. Interestingly,
171 treatment of patients using a combination of lymecycline (300 mg/d) and 5% BPO led to a
172 reduced proportion of type III within patients' samples, and a parallel reduction or
173 disappearance of their PMH lesions. In patients whose type III population was almost totally
174 eliminated there was almost no lesions remaining and the type distribution after treatment
175 generally reverted to that of controls (Fig. 1). Investigation of eight healthy female volunteers
176 also found that type III strains were more common on the back, especially the lower back, but
177 normally not present on the forehead or buccal mucosa; one patient was, however, found to
178 have a significant proportion of type III strains on their forehead despite, presumably, no
179 PMH lesions at this site (presence or absence of facial PMH was not definitely stated for this
180 patient). Unlike previous studies, a relatively high proportion of type III isolates was also
181 found on non-lesional skin, but this may have reflected issues around clear differentiation

between lesional and non-lesional sites using skin swab sampling as opposed to skin biopsy.

5. Challenges to the proposal that *C. acnes* is involved in the aetiology of PMH

5.1 PMH in the apparent absence of lesional *C. acnes*

Despite independent studies highlighting a strong association of *C. acnes* with PMH, a number of cases where *C. acnes* appears absent in lesional skin, as judged by Wood's lamp examination, histological staining and, in some cases, microbiological culture from skin swabs or biopsies have been reported[6,8,43-44]. It is important, however, to note that a negative Wood's lamp result does not confirm that *C. acnes* is absent, only that levels are below the density detection limit (~10^3 organisms)[45]. Furthermore, a recent investigation found that type II and III strains produce very low levels of porphyrin compared with type I organisms, and that cultures of type II and III strains on solid media do not fluoresce upon Wood's lamp illumination[46]. This indicates that lesions dominantly or solely colonised with type III strains may not have detectable follicular fluorescence. It also highlights that PMH lesions normally appear to have a mixed phylotype composition containing at least fluorescent type I strains, as well as type III in the majority of instances. The absence of mixed types and the detection of mostly type III strains based on previously described culture-based studies of lesional skin biopsies may, therefore, reflect the differential growth of dominant clones of this subspecies present in high numbers.

5.2 Rare occurrence of PMH on the face where *C. acnes* type III are normally absent.

A lack of facial involvement in PMH, despite high concentrations of *C. acnes* at this site, has been one of the biggest challenges to the view the bacterium has a role in the development of this condition. The observation of an association between *C. acnes* type III strains and PMH does, however, help to explain, at least in part, this intriguing clinical feature of the disease since type III strains normally appear to be absent or found in very low

206 abundance on the face of most individuals[38]. While a recent study reporting four adult cases
207 with apparent facial PMH, in addition to trunk, arm and leg lesions, appears inconsistent with
208 this view, no microbiological analysis was described for these patients[47]. As a result,
209 conclusions regarding the potential role of the bacterium in these specific facial cases of PMH
210 cannot be completely dismissed. It was interesting to note that the patients were much older
211 (40-65 years) than normally seen and it is currently unclear how the distribution of *C. acnes*
212 phylogroups and specific strain types on the skin may modify as we age; we can speculate
213 that in some older individuals type III strains may become more abundant on the face due to
214 age-related changes in cutaneous physiology that influence bacterial diversity. The previous
215 observation of a PMH patient with significant levels of type III on their forehead does
216 highlight that, while uncommon, the bacterium can indeed be present on the face of some
217 individuals[29] (section 4.2). However, the presumed presence of only truncal lesions on this
218 patient does complicate the view that a lack of facial PMH is solely down to the absence of
219 type III organisms at this skin site. Other factors, including the nature of the strain type(s)
220 present, their abundance and interaction with other microbiota, may well be important
221 factors, alongside host response and other variables.

222 **5.3 Antibacterial therapy versus phototherapy**

223 A number of studies have challenged the original findings of Relyeld et al.[25] in regards to
224 the effectiveness of antimicrobial treatment and phototherapy versus phototherapy alone. In
225 particular, Sim et al.[21] and Selim et al.[6] did not find any significant difference in
226 repigmentation of PMH lesions using daily topical 5% BPO and 1% clindamycin antimicrobial
227 treatments with NB-UVB versus NB-UVB monotherapy. Furthermore, in many cases
228 recurrence of the condition occurred, although some patients retained a degree of clinical
229 improvement. While such observations question the pathogenic role for *C. acnes* in PMH, a

key difference between these studies and that of Relyeld et al.[25] relates to the use of NB-UVB rather than UVA plus psoralen (PUVA). NB-UVB has been shown, *in vitro*, to have antibacterial effects on cutibacteria which is not observed with UVA, potentially explaining the contradictory results[48,49]. It is interesting to note, however, that in the study of Selim et al.[6] only two PMH patients had hypopigmented lesions that demonstrated fluorescence under a Wood's lamp indicating absent or low levels of *C. acnes*, or colonization with low porphyrin-producing strains, while data from Sim et al.[21] in relation to Wood's lamp analysis was not described. In contrast, Hassan et al.[9] found that topical (2% erythromycin lotion) and systemic (100 mg doxycycline b.i.d) antimicrobial treatments alongside NB-UVB for 3 months did give superior results compared to NB-UVB alone, and with no relapse in a 6 month follow up period.

6. Future research.

Additional studies are clearly needed to further dissect the underlying biological mechanisms driving the development of PMH. To date, our understanding of the underlying biology of type III strains and their interaction with other microbiota, alongside their niche requirements and capacity to cause disease, remains unclear, but an inflammatory phenotype has been observed, as well as the presence and absence of unique genomic elements when compared to other phylotypes[40,50]. It will be important to determine whether *C. acnes*, and particularly type III strains, have the capacity to interfere with melanogenesis via a depigmenting factor(s) or stimulation of a specific host response; initially, this could be achieved using appropriate *in vitro* cell culture models to study host-interacting properties. It is also interesting that some patients confuse PMH with leprosy, a chronic granulomatous disease caused by another intracellular bacterium, *Mycobacterium leprae*[1]. In particular, the tuberculoid from of the disease is characterised by a very small number of scaly, well defined

hypopigmented macules of varying symmetry on the skin, although poorly defined macules with mild hypopigmentation and erythema are also present in lepromatous forms[51]. Previous work has suggested this reflects marked reductions in the number of normal melanocytes in the lesions and the presence of atrophic melanocytes with reduced activity, while other studies suggest it may reflect defective transfer of melanosomes from melanocytes to keratinocytes[52,53]. In the tuberculoid form of the condition, acid-fast bacilli are rarely found, which may be a relevant observation when considering PMH lesions in the apparent absence of *C. acnes* (see section 5.1). While hypomelanosis disorders can also be caused by other types of microorganisms, such as fungi and yeasts, hypopigmentation in leprosy appears more noteworthy in the context of PMH given that mycobacteria and cutibacteria are distantly related actinomycetes, and have also been linked to another granulomatous disease, sarcoidosis[54]. While highly speculative, it may be that the hypopigmentation observed in both conditions is driven by some shared or similar characteristic of these bacteria (secretory or host-response). Previous studies on leprosy pathogenesis may, therefore, have informative aspects for researchers interested in future PMH studies, despite the many obvious differences between the two diseases.

The observation of PMH in twins, along with our current understanding of the epidemiology of the condition, also hints at a multifactorial inheritance aetiology driven by both genetics and environmental factors that may include specific strains of *C. acnes* and hormonal influences given its apparent increased rate in females and description of an acceleration of hypopigmentation in one patient after pregnancy[8]. The penetrance of PMH is, therefore, likely to be an interplay of these different influences, and further studies of genetic factors that may influence development of the disease and its clinical course should also be an important area of focus. We also need to better understand the pathophysiology of those

rare cases where PMH involves the face, as well as any differences that occur in the development of the condition in the presence and apparent absence of lesional *C. acnes*.

7. Conclusion

While a number of independent studies have found a strong association between *C. acnes* type III and PMH, a definite causal role is still to be determined. Nevertheless, the demonstration that type III strains are associated with the condition does help to explain, at least partly, the observations that PMH does not normally affect the face, nor is linked to the development of acne. Although reports of PMH in the apparent absence of *C. acnes* do complicate our understanding of the bacterium's role in the disease, further studies are required to definitely confirm this. It may be that *C. acnes* is only one of a number of different factors that can influence the development of PMH or that, in some instances, the bacterium initiates a biological response that leads to hypopigmentation, even after it becomes no longer detectable within lesions.

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Table 1. Key studies demonstrating an association between *C. acnes* and PMH based on culture analysis of lesional and adjacent non-lesional skin biopsies.

Study	M:F[a]	Biopsy	Lesional skin		Non-lesional skin		p-value[b]
			+	-	+	-	
Westerhof et al.	0:8	2 mm	7	1	1	7	0.04
Cavalcanti et al.	9:27	4 mm	33	2	4	31	<0.001
Total	**9:35**	**-**	**40**	**3**	**5**	**38**	<0.0001

[a]Male:Female ratio

[b]Statistical analysis was performed using McNemars test.

Table 2. Association of *C. acnes* AFLP genetic groups with acne and PMH

Disorder	AFLP analysis[a]			**Total**
	DNA group 1	DNA group 2	DNA group 3	
Acne	9	2	0	**11**
PMH	6	0	8	**14**
Total[b]	**15**	**2**	**8**	**25**

[a]Data taken from the study of Relyveld et al.[32]

[b]$p<0.01$ (Freeman-Halton extension of Fisher's exact test) for differences between acne and PMH in regards to DNA group distribution.

Table 3. Association of *C. acnes* phylotype and subspecies status with AFLP and other typing methods.

Phylotype	Subspecies	AFLP typing group[a]	*recA* typing phylotype	$MLST_8$ CC[b]	Ribotypes[c]
IA_1	*acnes*	1	IA_1/IB[d]	CC1; CC3; CC4	RT1; RT5; RT532
IA_2	*acnes*	1	IB	CC2	RT3; RT16
IB	*acnes*	1	IB	CC5	RT1
IC	*acnes*	1	IC	CC107	RT5
II	*defendens*	2	II	CC6; CC30; CC71, CC72	RT2; RT6
III	*elongatum*	3	III	CC77	RT9

[a]AFLP group from the study of Relyveld et al.[32]

[b]CC= clonal complex (https://pubmlst.org/cacnes/)

[c]Ribotypes based on the study of Fitz-Gibbon et al.[38]

[d] IA_1 = CC1 and CC3; IB = CC4.

FIGURE LEGENDS:

Figure 1. Clinical responses of PMH lesions to antimicrobial treatment. Lesional skin on the back of two patients before (a and c) and after (b and d) daily treatment with lymecycline (300 mg/d) and BPO washes for 3 months. Figure and modified legend are from Petersen et al[29].

Figure 2. Alignment of the 16S rDNA sequence from strain ATCC6919 (type IA_1), KPA171202 (type IB) and NCTC10390 (type II) versus type III isolates. The 16S rDNA G>A SNP described by Relyveld et al.[32] as a genetic marker of AFLP DNA group 3 strains is highlighted. This SNP was present in eight of the 10 type III isolates analysed, but absent in type strains from the other major *C. acnes* lineages. Figure and modified legend are from Barnard et al.[40]