

1 **Title:**

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

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## 22 **Abstract**

23 Progressive macular hypomelanosis (PMH) is a skin condition that normally causes  
24 symmetrically distributed hypopigmented macules on the front and back of the trunk, but  
25 rarely the face. To date, the pathophysiology of the condition is not well understood, but a  
26 role for the anaerobic skin bacterium *Cutibacterium* (previously *Propionibacterium*) *acnes* in  
27 the development of the disease has been proposed due to its sole presence within lesional,  
28 but not normal peri-lesional, skin. The success of antimicrobials in the treatment of PMH also  
29 provides circumstantial evidence that this association may be causal, although this is still to  
30 be proven. More recent culture and metagenomic typing studies indicate that strains of *C.*  
31 *acnes* subsp. *elongatum* (type III) may be important in the aetiology of the condition, which  
32 would help to explain why PMH does not normally affect the face since such strains are rarely  
33 present there, and why no association between this condition and acne vulgaris is found;  
34 acne appears to primarily involve type IA<sub>1</sub> strains from *C. acnes* subsp. *acnes* (type I). In this  
35 review we summarise current knowledge on the relationship between *C. acnes* and PMH, and  
36 re-examine previous challenges to the view that the bacterium plays a role in the condition  
37 against the backdrop of newly emerged data.

## 38 **1. Introduction**

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

## 58 **2. Histological and electron microscopic features**

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

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

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

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

#### 76 **3.1 Culture and non-culture-based detection**

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

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

### 93 **3.2 Therapeutic success of antimicrobial-based treatments**

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

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

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

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

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

##### 127 **4.1 Amplified Fragment Length Polymorphism and 16SrRNA gene analysis**

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

134 multiple bacterial colonies isolated from acne and PMH samples did not demonstrate the  
135 presence of mixed AFLP types. 16S rRNA gene sequencing revealed very high sequence  
136 identities between all clusters, with only a single nucleotide polymorphism (SNP) at position  
137 827 separating DNA groups I and II<sup>32</sup>, which is a characteristic difference between the well  
138 described *C. acnes* type I (*C. acnes* subsp. *acnes*) and type II (*C. acnes* subsp. *defendens*)  
139 phylotypes<sup>33</sup>, while group 3 isolates differed from group 1 due to a SNP at position 1243  
140 (G1243A). While biochemical analysis with the rapid ID 32A multi-test identification system  
141 confirmed DNA groups 1 and 2 as *C. acnes* (99.9% certainty), isolates from DNA group 3 gave  
142 ambiguous results and could not be identified phenotypically despite the molecular results  
143 indicating a unique *C. acnes* cluster; 16S rRNA identity is not, however, always a guarantee of  
144 species identity, especially in the case of a recently diverged and very closely related sister  
145 taxon<sup>34</sup>. This led to the proposal that organisms from DNA group 3 may represent a novel and  
146 very closely related bacterium from the genus *Cutibacterium*<sup>32</sup>.

#### 147 **4.2 PCR phylotyping and single- and multi-locus sequence type analysis**

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

156 Against this new landscape of understanding, and utilising the improved typing methods  
157 now available, Barnard et al.<sup>40</sup> 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*<sup>41,42</sup>) and lesions, but not those representing other  
161 phylogenetic groups, including those associated with acne (type IA<sub>1</sub>). 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<sup>32</sup>.

168 A subsequent study by Peterson et al.<sup>29</sup> 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



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

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

### 184 **5.1 PMH in the apparent absence of lesional *C. acnes***

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

### 200 **5.2 Rare occurrence of PMH on the face where *C. acnes* type III are normally absent.**

201 A lack of facial involvement in PMH, despite high concentrations of *C. acnes* at this site,  
202 has been one of the biggest challenges to the view the bacterium has a role in the  
203 development of this condition. The observation of an association between *C. acnes* type III  
204 strains and PMH does, however, help to explain, at least in part, this intriguing clinical feature  
205 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<sup>38</sup>. 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<sup>47</sup>. 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<sup>29</sup> (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.<sup>25</sup> in regards to  
224 the effectiveness of antimicrobial treatment and phototherapy versus phototherapy alone. In  
225 particular, Sim et al.<sup>21</sup> and Selim et al.<sup>6</sup> 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

230 key difference between these studies and that of Relyeld et al.<sup>25</sup> relates to the use of NB-UVB  
231 rather than UVA plus psoralen (PUVA). NB-UVB has been shown, *in vitro*, to have antibacterial  
232 effects on cutibacteria which is not observed with UVA, potentially explaining the  
233 contradictory results<sup>48,49</sup>. It is interesting to note, however, that in the study of Selim et al.<sup>6</sup>  
234 only two PMH patients had hypopigmented lesions that demonstrated fluorescence under a  
235 Wood's lamp indicating absent or low levels of *C. acnes*, or colonization with low porphyrin-  
236 producing strains, while data from Sim et al.<sup>21</sup> in relation to Wood's lamp analysis was not  
237 described. In contrast, Hassan et al.<sup>9</sup> found that topical (2% erythromycin lotion) and systemic  
238 (100 mg doxycycline b.i.d) antimicrobial treatments alongside NB-UVB for 3 months did give  
239 superior results compared to NB-UVB alone, and with no relapse in a 6 month follow up  
240 period.

## 241 **6. Future research.**

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

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

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

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

## 280 **7. Conclusion**

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

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430

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

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| Study             | M:F <sup>a</sup> | Biopsy | Lesional skin |          | Non-lesional skin |           | p-value <sup>b</sup> |
|-------------------|------------------|--------|---------------|----------|-------------------|-----------|----------------------|
|                   |                  |        | +             | -        | +                 | -         |                      |
| 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               |
| <b>Total</b>      | <b>9:35</b>      | -      | <b>40</b>     | <b>3</b> | <b>5</b>          | <b>38</b> | <b>&lt;0.0001</b>    |

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<sup>a</sup>Male:Female ratio

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<sup>b</sup>Statistical analysis was performed using McNemars test.

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

| Disorder                 | AFLP analysis <sup>a</sup> |             |             | Total     |
|--------------------------|----------------------------|-------------|-------------|-----------|
|                          | DNA group 1                | DNA group 2 | DNA group 3 |           |
| Acne                     | 9                          | 2           | 0           | <b>11</b> |
| PMH                      | 6                          | 0           | 8           | <b>14</b> |
| <b>Total<sup>b</sup></b> | <b>15</b>                  | <b>2</b>    | <b>8</b>    | <b>25</b> |

<sup>a</sup>Data taken from the study of Relyveld et al.<sup>32</sup>

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

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

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| Phylotype       | Subspecies       | AFLP typing group <sup>a</sup> | <i>recA</i> typing phylotype     | MLST <sub>8</sub> CC <sup>b</sup> | Ribotypes <sup>c</sup> |
|-----------------|------------------|--------------------------------|----------------------------------|-----------------------------------|------------------------|
| IA <sub>1</sub> | <i>acnes</i>     | 1                              | IA <sub>1</sub> /IB <sup>d</sup> | CC1; CC3; CC4                     | RT1; RT5; RT532        |
| IA <sub>2</sub> | <i>acnes</i>     | 1                              | IB                               | CC2                               | RT3; RT16              |
| IB              | <i>acnes</i>     | 1                              | IB                               | CC5                               | RT1                    |
| IC              | <i>acnes</i>     | 1                              | IC                               | CC107                             | RT5                    |
| II              | <i>defendens</i> | 2                              | II                               | CC6; CC30; CC71, CC72             | RT2; RT6               |
| III             | <i>elongatum</i> | 3                              | III                              | CC77                              | RT9                    |

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<sup>a</sup>AFLP group from the study of Relyveld et al.<sup>32</sup>

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<sup>b</sup>CC= clonal complex (<https://pubmlst.org/cacnes/>)

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<sup>c</sup>Ribotypes based on the study of Fitz-Gibbon et al.<sup>38</sup>

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<sup>d</sup>IA<sub>1</sub> = CC1 and CC3; IB = CC4.

470 **FIGURE LEGENDS:**

471

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

476

477 **Figure 2. Alignment of the 16S rDNA sequence from strain ATCC6919 (type IA<sub>1</sub>),**  
478 **KPA171202 (type IB) and NCTC10390 (type II) versus type III isolates.** The 16S rDNA G>A  
479 SNP described by Relyveld et al.<sup>32</sup> as a genetic marker of AFLP DNA group 3 strains is  
480 highlighted. This SNP was present in eight of the 10 type III isolates analysed, but absent in  
481 type strains from the other major *C. acnes* lineages. Figure and modified legend are from  
482 Barnard et al.<sup>40</sup>