An increased incidence of *Propionibacterium acnes* biofilms in acne vulgaris: a case–control study

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Summary

Background Acne vulgaris is a disorder of the sebaceous follicles. *Propionibacterium acnes* can be involved in inflammatory acne.
Objectives This case–control study aimed at investigating the occurrence and localization of P. acnes in facial biopsies in acne and to characterize the P. acnes phylotype in skin compartments.
Methods Specific monoclonal and polyclonal antibodies were applied to skin biopsies of 38 patients with acne and matching controls to localize and characterize P. acnes and to determine expression of co-haemolysin CAMP factor, a putative virulence determinant.
Results Follicular P. acnes was demonstrated in 18 (47%) samples from patients with acne and eight (21%) control samples [odds ratio (OR) 3·37, 95% confidence interval (CI) 1·23–9·23; P = 0·017]. In 14 (37%) samples from patients with acne, P. acnes was visualized in large macrocolonies/biofilms in sebaceous follicles compared with only five (13%) control samples (OR 3·85, 95% CI 1·22–12·14; P = 0·021). Macrocolonies/biofilms consisting of mixed P. acnes phylotypes expressing CAMP1 were detected in both case and control samples. Only four samples tested positive for the presence of *Staphylococcus* spp. and fungi were not observed.
Conclusions We have for the first time visualized different P. acnes phylotypes in macrocolonies/biofilms in sebaceous follicles of skin biopsies. Our results support the hypothesis that P. acnes can play a role in the pathogenesis of acne as acne samples showed a higher prevalence of follicular P. acnes colonization, both in terms of follicles containing P. acnes and the greater numbers of bacteria in macrocolonies/biofilms than in control samples.

Acne vulgaris is a common inflammatory disorder of the sebaceous follicles, affecting more than 80% of young adolescents, but can also persist into adulthood.[1] The mechanisms by which inflammatory lesions arise are poorly understood but bacterial involvement has been implicated.[2,3]

Propionibacterium acnes is a Gram-positive pleomorphic rod and is traditionally regarded as part of the normal human skin microbiota. *Propionibacterium acnes* strains are categorized as phylotypes IA, IB, II and III according to sequence comparison of their *tly* and *recA* genes.[4,5] The entire genomic sequence of P. acnes reveals numerous genes with host-degrading properties.[6] Particularly, P. acnes carries five genes encoding cohaemolytic CAMP (Christie–Atkins–Munch–Peterson) factor

homologues[7] which have been hypothesized to contribute to bacterial virulence.[8]

The evidence that P. acnes can play a major part in the inflammatory reaction accompanying acne vulgaris is largely circumstantial.[9–11] A recent comprehensive review has suggested that P. acnes neither initiates comedogenesis nor has a role in the initiation of inflammation in inflamed acne lesions.[12] The major limitation of studies attempting to elucidate the role of P. acnes in acne vulgaris is their reliance on bacterial culture with various sampling techniques.[11,13,14] The bacterial culture cannot reliably distinguish between P. acnes populations with presumably different pathogenic potential, e.g. epidermal and follicular. *Propionibacterium acnes* can form

biofilms in vitro[15,16] as well as in connection with implant[17] and prostate colonization[18] and is presumed to be able to establish biofilm in acne vulgaris. Biofilm-containing tissues require special techniques for both bacterial sampling and culture.[17,19,20] To the best of our knowledge these techniques have not been applied in acne vulgaris. In contrast, direct visualization of bacteria with immunofluorescence microscopy (IFM) assay enables visualization and identification of individual cells within diseased tissue.

In this paper we report an observational case–control study investigating the occurrence and localization of *P. acnes* in facial skin biopsies from patients with acne. We further characterized *P. acnes* populations by applying specific staining for *P. acnes* type IA and type II as well as CAMP factor.

Materials and methods

Patients

The case group comprised 38 patients with acne vulgaris. Twenty males and 18 females with a median age of 19 years (range 15–63) were studied. Twenty-nine patients were included in a Lithuanian prospective study. These patients with acne had received neither topical treatment for 2 weeks, nor systemic treatment for 1 month, prior to biopsy. Acne severity score was assigned in 29 patients from Lithuania, median 3 (range 2–5). Biopsies from nine patients with acne were identified at the biobank of Umeå University Hospital, Umeå, Sweden. No information on preceding or concurrent acne treatment was available for these patients. In total, 38 lesional skin samples (six cases of comedones and 32 cases of inflammatory lesions) were studied.

The control group was selected from a cohort of patients who underwent benign naevus incision (Västerbotten County, Sweden). For each case, one control was randomly selected matched on age, sex and biopsy location. The control subjects included in the study had no records on concurrent acne in medical charts, although mild acne might have been undocumented. The control skin samples were then retrieved from the biobank of Umeå University Hospital, Umeå, Sweden.

Only facial (either punch or incisional) biopsies were studied. The skin specimens were fixed, paraffin-embedded and stored at room temperature pending investigation. The biopsy material from control subjects was divided into two or three pieces. The pieces containing minimal or no naevus lesions were selected for further analysis. The number of sebaceous follicles was calculated in every biopsy.

Written informed consent was obtained from all patients enrolled in this study, which was conducted according to the ethical standards of the Lithuanian Bioethical Committee and the University of Umeå.

Immunolabelling

Immunofluorescence microscopy

Primary antibodies used in the study are listed in Table 1.[5,7,21,22] After antigen retrieval, skin samples were treated with the respective primary antibody followed by treatment with an appropriate fluorochrome-labelled conjugate (Life Technologies, NY, USA). All samples were screened with monoclonal antibodies known to react with either *P. acnes* or *Staphylococcus* spp. Selected samples were subjected to double simultaneous staining. In this scenario, skin samples were stained with a monoclonal antibody (anti-type IA, anti-type II, anti-CAMP1) and polyclonal *P. acnes* rabbit antibody followed by incubation with labelled secondary antibodies.

All immunolabelled samples were also stained with 4′,6′-diamidino-2-phenylindole (DAPI), which detects the DNA of both bacterial and eukaryotic cells.

Microscopy

The slides were analysed using an Axioplan 2 imaging microscope (Carl Zeiss, Oberkochen, Germany). Selected samples were imaged using a confocal laser scanning microscope (Leica Microsystems, Wetzlar, Germany).

Table 1 Antibodies used for immunofluorescence microscopy

Antibody	Specificity	Target	Reference
Mouse monoclonal (QUBPa1)	Type IA *Propionibacterium acnes*	Dermatan sulphate-binding protein	McDowell *et al.* 2005[5] McDowell *et al.* 2011[21]
Mouse monoclonal (QUBPa2)	Type II *Propionibacterium acnes*	Carbohydrate/glycolipid components of cell envelope	McDowell *et al.* 2005[5]
Mouse monoclonal (QUBPa4)	CAMP factor 1	CAMP factor 1	Valanne *et al.* 2005[7]
Mouse monoclonal (QUBPa3)	*Propionibacterium acnes* IA, IB, II, III, *P. granulosum*	Surface exposed antigen containing carbohydrate/glycolipid	Tunney *et al.* 1999[22]
Mouse monoclonal (QUBSe1)	*Staphylococcus* spp., including *S. aureus*	Lipoteichoic acid	A. McDowell & S. Patrick, unpublished data
Rabbit polyclonal	As QUBPa3 exclusive *Propionibacterium granulosum*	Multiple surface antigens	A. McDowell & S. Patrick, unpublished data

Statistics

The association between the presence of P. *acnes* and acne vulgaris was assessed by estimate of the odds ratio (OR) and corresponding 95% confidence interval (CI). Two-tailed *t*-tests were also used.

Results

Presence of *Propionibacterium acnes* in facial skin biopsies

Facial skin biopsies from 38 patients with acne and matching control subjects were analysed in this study. The biopsies contained a range of one to eight sebaceous follicles per sample. One case and two control samples contained no sebaceous follicles.

Propionibacterium acnes was detected by IFM microscopy in 19 patients with acne and nine control subjects. Eighteen patient biopsies (47%) contained P. *acnes* in sebaceous follicles, as compared with only eight control biopsies (21%) (OR 3·37, 95% CI 1·23–9·23; P = 0·017) (Table 2). Two subjects (one patient with acne and one control subject) had P. *acnes* exclusively in the stratum corneum. There were more males in P. *acnes*-positive controls (male/female ratio 3 : 1) than in P. *acnes*-positive patients with acne (male/female ratio 1 : 1·25).

We observed P. *acnes* either (i) as macrocolonies of > 1000 bacterial cells which formed extensive biofilms, which we defined according to the modified criteria of Parsek and Singh, namely surface association, confined localization of bacteria, and occurrence of matrix-encased bacterial cell clusters,[23,24] or (ii) as microcolonies of < 100 bacterial cells. According to these criteria, 14 patients with acne (37%) showed large P. *acnes* macrocolonies/biofilms in sebaceous follicles compared with only five (13%) positive control samples (OR 3·85, 95% CI 1·22–12·14; P = 0·021). In two of the patient samples P. *acnes* macrocolony/biofilm was observed in both sebaceous follicle as well as within the stratum corneum. Bacteria were not observed on the stratum corneum surface as superficial colonies were washed away during processing. Except for a cluster of 12 P. *acnes* bacteria in one sebaceous gland, the skin appendages (sebaceous glands, eccrine sweat glands) have universally been negative in P. *acnes*/Staphylococcus spp./DAPI staining.

We then considered the possibility that the higher detection rate of P. *acnes* in the acne group could be attributed to a higher density of sebaceous follicles. However, biopsies from patients with acne had a lower number of sebaceous follicles (median 1, range 0–5) than control samples (median 4, range 0–8) (P = 0·0001, paired Student *t*-test).

We selected samples with at least two sebaceous follicles to test whether P. *acnes* had a focal distribution in the skin samples and which portion of sebaceous follicles was colonized by the bacteria. We calculated the number of sebaceous follicles in all patients and controls enrolled in the study. Of these, 17 (45%) acne biopsies and 33 (87%) control biopsies

Table 2 Pattern of follicular *Propionibacterium acnes* colonization in patients with acne and control subjects

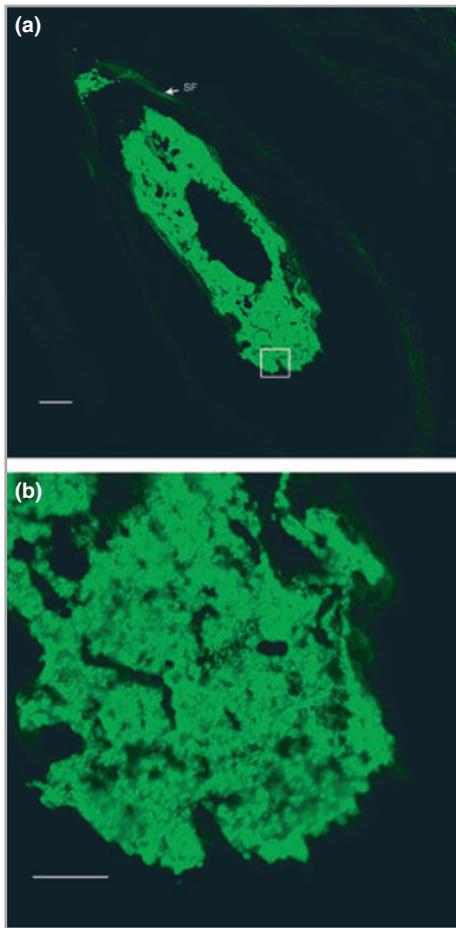| Cases (n = 18)			Controls (n = 8)		
Total number of sebaceous follicles	Number of sebaceous follicles with macrocolony/biofilm	Number of sebaceous follicles with microcolonies	Total number of sebaceous follicles	Number of sebaceous follicles with macrocolony/biofilm	Number of sebaceous follicles with microcolonies
1		1	2	1	
4	1	1	5	1	
1	1		7	2	2
1	1		5		1
2	1		7	2	2
1	1		2	2	
2	1		3		1
1	1		6		2
1		1			
1	1[a]				
1	1				
5	2[a]				
3	1				
4	3				
1	1				
3	1				
1		1			
5		1			

[a]Biofilm within stratum corneum.

Fig 1. Confocal laser scanning microscope image of a skin biopsy from a patient with acne labelled with *Propionibacterium acnes*-specific monoclonal antibody and Alexa®488 conjugated anti-mouse secondary antibody (green). A *P. acnes* macrocolony/biofilm in a sebaceous follicle (a): the arrow indicates the boundary of the sebaceous follicle (SF), scale bar 20 μm. Close-up (b) of the area marked in (a), scale bar 10 μm.

Fig 2. Confocal laser scanning microscope image of an acne skin biopsy labelled with *Propionibacterium acnes*-specific polyclonal antibody and Alexa®555 conjugated anti-rabbit secondary antibody (red). The purple autofluorescence of the hair was captured using 668 nm channel. *Propionibacterium acnes* microcolonies attached to a hair shaft (a): the arrows indicate hair shafts (HS), scale bar 20 μm. Close-up (b) of the area marked in (a): the arrows indicate *P. acnes* cells, scale bar 5 μm.

contained at least two sebaceous follicles. In every individual skin biopsy we calculated the total number of sebaceous follicles and the number of P. *acnes*-positive sebaceous follicles. A median of 36·5% (range 20–75%) and 41·5% (range 20–100%) of sebaceous follicles was positive for P. *acnes* in acne and control biopsies, respectively.

In order to obtain further insights into P. *acnes* colonization of individual skin samples, sequential sections from the same tissue were evaluated. Up to 15 4-μm thick sections were subjected to IFM in selected acne and control cases. A few notable observations were made. (i) Up to a few hundreds of thousands P. *acnes* colonized an individual sebaceous follicle (Fig. 1, Video S1; see Supporting Information). (ii) *Propionibacterium acnes* macrocolony/biofilm penetrated deep in the sebaceous follicle as evidenced by association of the bacteria with the inner root sheath. (iii) *Propionibacterium acnes* colonies were attached to the hair shaft (Fig. 2). (iv) *Propionibacterium acnes*
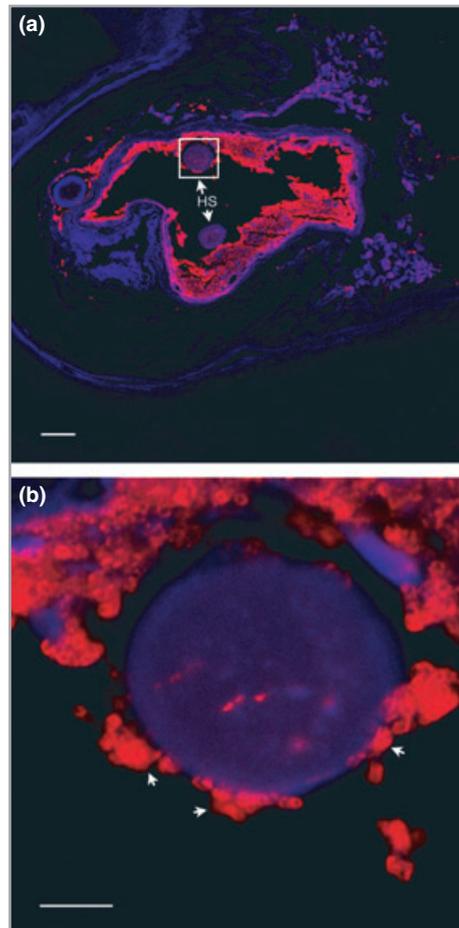
showed a highly focal distribution in the stratum corneum with P. *acnes* cells separated by 4–12 μm.

Distribution of different phylotypes of *Propionibacterium acnes* in acne and control samples

Nine acne and nine control samples were subjected to double staining using monoclonal antibodies that react with either P. *acnes* type IA or type II together with a polyclonal rabbit anti-P. *acnes* antibody, reactive with both types, to determine if there was a predominant P. *acnes* phylotype. Both type IA and type II were observed within the same sebaceous follicles and the same macrocolonies/biofilms (Fig. 3a). The semiquantitative analysis of selected sebaceous follicles with mixed P. *acnes* populations revealed a predominant (> 2/3 of total biomass) type II infection. Contrary to the sebaceous follicle, the stratum corneum macrocolonies/biofilms were positive only
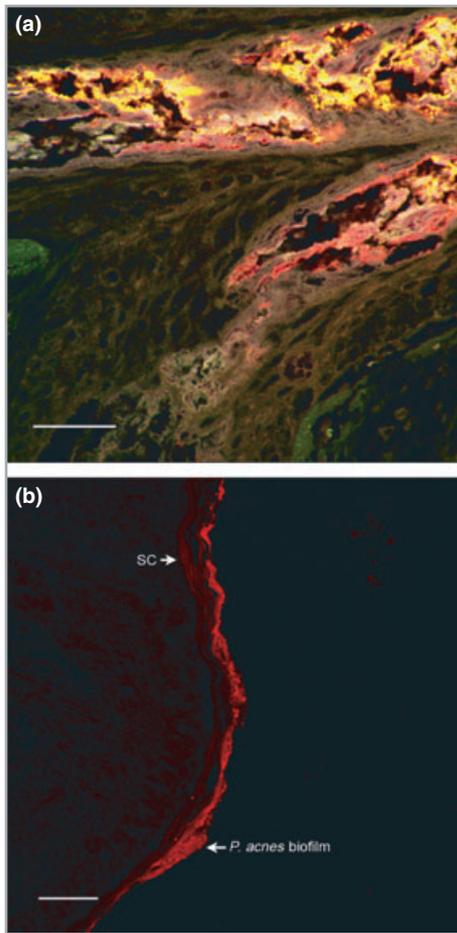
Fig 3. (a) Confocal laser scanning microscope (CLSM) image of an acne sample labelled with *Propionibacterium acnes* type II-specific monoclonal antibody/Alexa®488 conjugated anti-mouse secondary antibody (green) and P. *acnes*-specific polyclonal antibody/Alexa®555 conjugated anti-rabbit secondary antibody (red). The image shows a mixed population of P. *acnes* phylotypes in the same sebaceous follicle. Double-labelled bacteria appear yellow in the image, scale bar 50 μm. (b) CLSM image of a control skin biopsy labelled with P. *acnes* type II-specific monoclonal antibody and Alexa®555 conjugated anti-mouse secondary antibody (red): P. *acnes* biofilm within the stratum corneum (SC), scale bar 50 μm.

Fig 4. Confocal laser scanning microscope image of a sebaceous follicle of a control sample labelled with *Propionibacterium acnes* type IA-specific monoclonal antibody/Alexa®488 conjugated anti-mouse secondary antibody (green) and P. *acnes*-specific polyclonal antibody/Alexa®555 conjugated anti-rabbit secondary antibody (red). *Propionibacterium acnes* labelled with polyclonal antibody (a), scale bar 5 μm. Overlay image of double-labelled P. *acnes* (b), scale bar 5 μm. Note extracellular and surface-associated labelling with anti-dermatan sulphate-binding protein P. *acnes* type IA antibody. Arrow indicates extracellular staining of dermatan sulphate-binding protein.

for type II P. *acnes* phylotype (Fig. 3b, Video S2; see Supporting Information). Extracellular type IA antigen was observed in the vicinity of P. *acnes* (Fig. 4).

CAMP1 staining

Six biofilm-positive samples (three acne and three control) were subjected to double labelling with anti-CAMP1 monoclonal and anti-whole cell P. *acnes* polyclonal antibodies. A varied cell surface-associated CAMP1 staining was seen in follicular P. *acnes* populations in all samples (Fig. 5a, b). Moreover, a proportion of epidermal P. *acnes* population in a control sample also showed a bacterial cell surface-associated labelling (Fig. 5c, d).
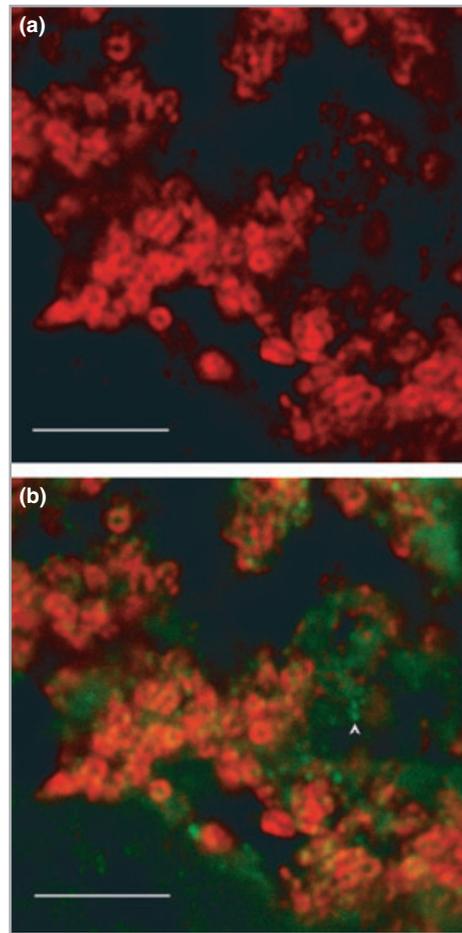
Presence of other microbes in facial skin biopsies

In order to evaluate if other microbes could be visualized in sebaceous follicles, all acne and control biopsies were subjected to IFM with an anti-*Staphylococcus* spp. monoclonal antibody as well as DAPI staining. One of the acne patient biopsies and three of the control biopsies were positive for *Staphylococcus* spp. One control biopsy was positive for both organisms in the same sebaceous follicle. One of the positive control samples showed also DAPI positive but P. *acnes* and *Staphylococcus* spp. negative organisms. Approximately 100 coccoids in the upper part of a sebaceous follicle were noted. In our experience, DAPI serves as an excellent surrogate marker to visualize fungi due to their characteristic morphology. All sebaceous follicles investigated were negative for fungal species.
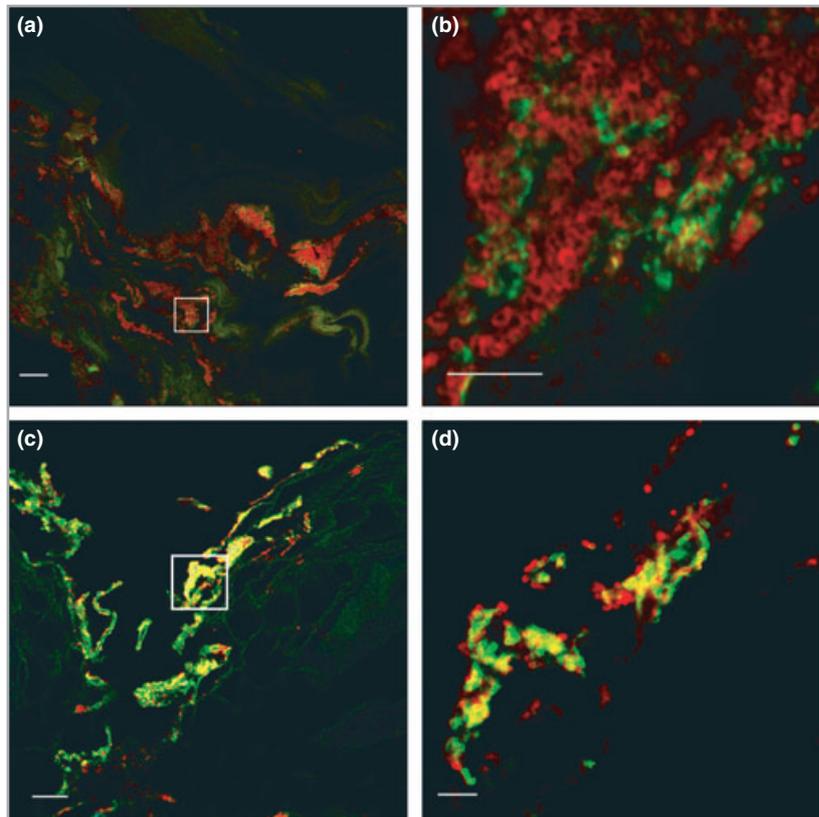
Fig 5. Confocal laser scanning microscope images of skin biopsies labelled with CAMP1-specific monoclonal antibody/Alexa®488 conjugated anti-mouse secondary antibody (green) and *Propionibacterium acnes*-specific polyclonal antibody/Alexa®555 conjugated anti-rabbit secondary antibody (red). Double-labelled bacteria appear yellow. Variable surface-associated CAMP labelling of bacteria in a sebaceous follicle of an acne sample (a), scale bar 20 µm. Close-up (b) of the area marked in (a), scale bar 5 µm. Epidermal population of P. acnes showing bacterial cell surface labelling in a control sample (c), scale bar 20 µm. Close-up (d) of the area marked in (c), scale bar 5 µm.

Discussion

We visualized P. acnes in facial skin biopsies from 38 patients with acne and sex- and age-matched controls. Our data indicate that P. acnes colonizes sebaceous follicles in acne facial lesions more often than in controls. In sebaceous follicles P. acnes was, as a rule, associated with a follicle epithelium and existed as a large cell aggregate which appeared to be encased in a matrix. These features have been set as criteria for biofilm definition.[24]

A recent comprehensive review of microbial aetiology of acne[12] along with others[9,25] has proposed that P. acnes has no role in the initiation of inflammation in inflamed acne lesions. The key arguments refer to the findings of P. acnes in both acne-affected and normal sebaceous follicles. Two major techniques were applied to retrieve P. acnes from the skin for subsequent culture: punch biopsy and cyanoacrylate gel extraction. The punch biopsy targets both epidermal as well as the follicular populations of P. acnes. Introducing a needle through an overlying skin containing bacteria could seed bacteria along the needle track into deeper parts of the skin. A recent elegant study of Grice *et al.*[26] showed that similar microbial populations are captured by swab, scrape or punch biopsy. An abundance-based Jaccard similarity index value for comparison between scrape and punch was 1·0, suggesting that the microbial populations collected were essentially the same. The cyanoacrylate gel application is used to retrieve P. acnes from hair follicle infundibulum.[27] However, its ability

to retrieve P. acnes macrocolonies/biofilms is less certain. Bacterial biofilms are known to become irreversibly adherent to underlying surfaces.[28] Even scraping with a surgical blade has failed to detach P. acnes biofilm from metallic surfaces *in vitro*.[29] Moreover, our study demonstrated that P. acnes macrocolony/biofilm can penetrate deep in the sebaceous follicle and attach to the hair shaft. These populations can also be undetectable by cyanoacrylate gel application. Moreover, the cyanoacrylate biopsy removes a varying amount of stratum corneum.[30] Both the sampling techniques (punch and cyanoacrylate biopsy) target epidermal and follicular P. acnes populations while IFM provides direct visualization of P. acnes in sebaceous follicles.

While the direct visualization of P. acnes is clearly advantageous for the identification of individual cells in skin biopsies, a few limitations warrant mentioning. The number of sebaceous follicles in individual biopsies can vary and, therefore, influence the P. acnes detection rate. Thirteen (65%) of P. acnes-negative acne and three (10%) of P. acnes-negative control biopsies had only one sebaceous follicle in the sample. This in turn may indicate that the detection rate of P. acnes in acne biopsies is underestimated. Overall, control biopsies had a significantly higher sebaceous follicle number than acne biopsies, thus further strengthening the observed higher ratio of P. acnes in acne biopsies.

Propionibacterium acnes is known to form biofilms both *in vitro* and *in vivo*,[15–17] and the P. acnes macrocolonies we observed in the sebaceous follicles are likely to represent matrix-encased

biofilms. We showed that P. acnes macrocolonies/biofilms are encountered more often in acne-affected sebaceous follicles than in control samples. Further characterization of the P. acnes macrocolony/biofilm was achieved by visualizing P. acnes IA and II phylotypes. Our results indicate that P. acnes macrocolonies/biofilms in sebaceous follicles consist of at least two different phylotypes (IA and II). Due to the absence of reliable markers for P. acnes IB and III phylotypes and P. granulosum, their presence could not be excluded. A small proportion of bacteria failed to label with either the type IA or type II specific monoclonal antibodies. These may therefore belong to a different P. acnes phylotype or other related species as the common P. acnes antibody expresses a weak cross-reactivity with P. granulosum (A.C. Jahns, personal observation) as well as Actinomyces spp. (S. Patrick, personal observation). The presence of mixed P. acnes phylotypes IA and II within the same macrocolony/biofilm in an individual sebaceous follicle makes it difficult to associate either phylotype specifically with the aetiology of acne.[31]

Notably, type IA labelling revealed a fine extracellular staining in the vicinity of P. acnes type IA, presumably in the extracellular polymeric substance of biofilms. The type IA monoclonal antibody targets a dermatan sulphate-binding protein.[21] The protein is known to be secreted by P. acnes[32] and possesses immunoreactive properties.[33] Other bacterial biofilms are reported to contain bacterial proteins in the associated matrix.[34,35]

As with other bacterial pathogens,[36,37] the production of a cohaemolytic CAMP factor has been hypothesized to play a pathogenic role in P. acnes.[8,38] Moreover, a recombinant CAMP factor has induced local inflammation when injected in mouse ear skin.[38] Although limited by small sample numbers, our study demonstrated a P. acnes CAMP1 expression in both case and control samples, as well as in follicular and epidermal populations. This may be indicative of common CAMP expression in both inflamed and noninflamed sebaceous follicles. Whether CAMP does indeed contribute to P. acnes-induced inflammation in acne vulgaris needs further study.

Few biopsies showed the presence of P. acnes within the stratum corneum. This observation appears to contradict earlier studies reporting a nearly 100% P. acnes isolation rate using swab or scrape collection techniques.[11,13,39] While a classical swab collection approach targets a 4-cm^2 skin area,[40] a range of 20–40 mm^2 skin surface is targeted in biopsy material. Moreover, the immunolabelling only targets P. acnes situated inside the stratum corneum while superficial colonies are washed away during the sample preparation. Propionibacterium acnes demonstrated within the stratum corneum represent live bacteria as they were hybridized with 16S and 23S rRNA probes (D.A. Alexeyev, personal observation). The detection rate of epidermal P. acnes populations using visualization techniques is, therefore, clearly underestimated. In order to obtain further insights into the pattern of P. acnes distribution we studied up to 15 sequential sections from the same biopsies. In both acne and control biopsies P. acnes affected only a number of sebaceous follicles, reflecting a highly uneven distribution of P. acnes sebaceous follicle colonization. The distribution was also highly uneven within the stratum corneum, with bacterial clusters separated by 4–16 µm.

The commonly reported presence of Staphylococcus spp. and Malassezia in sebaceous follicles has been used as an argument against a role for P. acnes in acne inflammation.[13,41] Follicular populations of Staphylococcus spp. and fungal species were studied by applying IFM and DAPI staining, respectively. Few samples scored positive for Staphylococcus spp. and none for fungi. These data indicate that both species are either not present or are present in too negligible a load to play a significant role in sebaceous follicle inflammation.

In conclusion, our case–control study, based on direct visualization of P. acnes in skin biopsies, has demonstrated a statistically significant association of follicular P. acnes populations with the development of acne vulgaris. Our data do not support the notion that P. acnes is a ubiquitous inhabitant of sebaceous follicles as only 20% of control subjects were positive. Moreover, extensive P. acnes macrocolonies/biofilms are a more common phenomenon in acne vulgaris than in controls. We observed highly complex P. acnes macrocolonies/biofilms composed of different P. acnes phylotypes which contained secreted bacterial proteins with known immunoreactive properties. We have not found any qualitative differences between P. acnes macrocolonies/biofilms in acne and control samples as both case and control follicular P. acnes macrocolonies/biofilms showed expression of dermatan sulphate-binding protein and CAMP1 factor. This may indicate that phenotypic, rather than genetic, changes associated with biofilm formation may account for the pathogenic role of P. acnes in acne vulgaris. While further prospective study of patients and controls using these exquisitely sensitive tools is necessary to determine the detail of the interactions of P. acnes within the skin, our findings do not rule out the possibility, and indeed suggest, that P. acnes may play a role in the pathogenesis of acne vulgaris.

What's already known about this topic?

- Acne vulgaris is an inflammatory disorder.
- The role of *Propionibacterium acnes* in acne pathogenesis is controversial.
- *Propionibacterium acnes* is suggested to play a role in acne inflammation.

What does this study add?

- *Propionibacterium acnes* represents a dominant microorganism in acne vulgaris and occurs more often than in controls.
- *Propionibacterium acnes* forms large macrocolonies/biofilms in sebaceous follicles which consist of different P. acnes phylotypes and secreted bacterial proteins.

Acknowledgments

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Supporting Information

Additional Supporting Information may be found in the online version of the article:

Video S1. Three-dimensional (3D) reconstruction of Figure 1 with *Propionibacterium acnes* biofilm in the sebaceous follicle. *Propionibacterium acnes* is labelled with common monoclonal antibody and Alexa®488 conjugated antimouse secondary antibody (green). 3D volumetric confocal laser scanning microscope images were reconstructed from a series of horizontal-sectional images using commercial software LAS AF (Leica Microsystems, Wetzlar, Germany).

Video S2. Three-dimensional (3D) reconstruction of Figure 3b with *Propionibacterium acnes* biofilm within the stratum corne-um. *Propionibacterium acnes* is labelled with type II specific monoclonal antibody and Alexa®555 conjugated antimouse secondary antibody (red). 3D volumetric confocal laser scanning microscope images were reconstructed from a series of horizontal-sectional images using commercial software LAS AF (Leica Microsystems, Wetzlar, Germany).

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