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SYSTEMATIC REVIEW AND META–ANALYSIS

A systematic literature review of the human skin microbiome as biomarker for dermatological drug development

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AIMS

To explore the potential of the skin microbiome as biomarker in six dermatological conditions: atopic dermatitis (AD), acne vulgaris (AV), psoriasis vulgaris (PV), hidradenitis suppurativa (HS), seborrhoeic dermatitis/pityriasis capitis (SD/PC) and ulcus cruris (UC).

METHODS

A systematic literature review was conducted according to the PRISMA guidelines. Two investigators independently reviewed the included studies and ranked the suitability microbiome implementation for early phase clinical studies in an adapted GRADE method.

RESULTS

In total, 841 papers were identified and after screening of titles and abstracts for eligibility we identified 42 manuscripts that could be included in the review. Eleven studies were included for AD, five for AV, 10 for PV, two for HS, four for SD and 10 for UC. For AD and AV, multiple studies report the relationship between the skin microbiome, disease severity and clinical response to treatment. This is currently lacking for the remaining conditions.

CONCLUSION

For two indications – AD and AV – there is preliminary evidence to support implementation of the skin microbiome as biomarkers in early phase clinical trials. For PV, UC, SD and HS there is insufficient evidence from the literature. More microbiome-directed prospective studies studying the effect of current treatments on the microbiome with special attention for patient meta-data, sampling methods and analysis methods are needed to draw more substantial conclusions.

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Introduction

The escalating number of therapeutic candidates in drug development programs require strategies that optimize the process of clinical development. A common approach is the use of biomarkers in clinical trials. A biomarker is defined as a characteristic that can be objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention [1, 2]. Clinical biomarkers are thought to reflect disease activity and pathophysiology [3, 4]. A useful biomarker in any class has to comply with the following general criteria: (i) there must be a consistent response of the biomarker across studies (preferably from different research groups) and drugs from the same mechanistic class; (ii) the biomarker must respond clearly to therapeutic (not supratherapeutic) doses; (iii) there must be a clear dose- or concentration-response relationship; and (iv) there must a plausible relationship between the biomarker, pharmacology of the drug class and disease pathophysiology [4]. Validated biomarkers are often being used to guide drug development programmes from human pharmacology studies, i.e. phase 1 trials, to confirmatory trials, i.e. phase 3 studies [2]. For dermatological diseases the drug developers often rely on clinical efficacy scores, e.g. the Eczema Area and Severity Index (EASI) for atopic dermatitis (AD), Psoriasis Area and Severity Index for psoriasis vulgaris (PV) and inflammatory lesion count for acne vulgaris (AV) or investigator global assessments. However, more objective outcome measures including validated biomarkers would have great added value in this field. One of these potential new biomarkers is the human skin microbiome, which has the potential to monitor disease activity and drug specific (mechanistic) effects.

The human microbiome refers to the combined genomic information of all microbial communities living on or in the human body. Collectively, this encompasses fungi (mycobiota), bacteria (microbiota), viruses, bacteriophage, archaea and protozoa. This, along with the human genome, completes what is now termed the human microbial superorganism [5]. The skin microbiome harbours vast microbial communities living in a range of both physiologically and topographically distinct niches and microenvironments [6, 7]. Actinobacteria (52%), Firmicutes (24%), Proteobacteria (17%) and Bacteroidetes (7%) are the four most abundant species identified on the skin [8]. Previous studies have shown that it is not only skin topography that influences microbial colonization, but also a vast range of host-specific factors including age and sex, and environmental factors such as occupation, clothing choice, antibiotic use, cosmetics, soaps, environmental temperature, humidity, and longitudinal and/or latitudinal variation in UV exposure, which can all contribute to the variability seen in the microbial flora of the skin [9–15]. Moreover, changes or aberrations in the skin microbiome have been implicated in the pathophysiology of numerous skin diseases such as AD and AV [16].

Several reviews have described the role and impact of skin microbiome on disease [17–22]. However, to date, no structured review has been conducted to evaluate the feasibility, suitability and potential use of the skin microbiome as biomarker for early phase clinical drug development. Therefore, we conducted a systemic literature review with predefined search terms according to the PRISMA guidelines, with focus on six relevant disorders, i.e. AD, seborrhoeic dermatitis and pityriasis capitis (dandruff; SD/PC), AV, hidradenitis suppurativa (HS), PV and ulcus cruris/chronic wounds (UC). In addition, we evaluated and ranked the conditions regarding the potential as clinical biomarker. Lastly, we provided recommendations for prospective microbiome investigations in clinical drug development programmes.

Methods

We followed the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) [23]. In collaboration with a trained librarian from the Leiden University Medical Centre, a structured electronic literature search was composed, using a combination of two main search criteria: microbiome and the targeted skin condition (i.e. AD, SD/PC, HS, AV, UC and PV). For each search term, all relevant keyword variations were used in conjunction with free text word variations. The search strategy was optimized for all consulted databases, taking into account the differences of the various controlled vocabularies, as well as the differences of database-specific technical variations (e.g. the use of quotation marks). The final search was performed on 29 September 2017, using bibliographic databases including PubMed (incl. MEDLINE), Embase (OVID-version), Web of Science, Cochrane Library, CENTRAL, Academic Search Premier and ScienceDirect. Animal-only studies, reviews without original data, non-English studies and case studies were excluded. Moreover, culture-based methods were excluded since the objective of this review was to explore the full microbiome profile and relative abundances compared to other genus as biomarker. The remaining studies were fully reviewed. The overall quality of evidence was rated using pre-defined criteria (group size, type of control, method of sampling, serial sampling available, well defined metadata, analysis method). *Grading of Recommendations Assessment, Development and Evaluation* (GRADE) guidelines were used as guidance for rating the quality of evidence [24]. This was done by two investigators independently and the final outcome was determined by discussion once discrepancies occurred.

Results

The search resulted in 841 titles. After duplicates were removed, 443 papers were screened for inclusion. Four-hundred-and-one manuscripts were excluded based on the exclusion criteria with mostly culture-based studies that were not eligible. The remaining 42 studies were identified as using nonculture-based methods to analyse microbiome populations in one of the targeted skin conditions and fully reviewed, Figure 1. All 42 were included in the review, the study characteristics can be found in Table 1.

Psoriasis vulgaris

In 10 studies, the cutaneous microbiome in PV patients was investigated, Table 1 [25–34]. In addition to microbiota, these studies have focused on the mycobiota. An increased

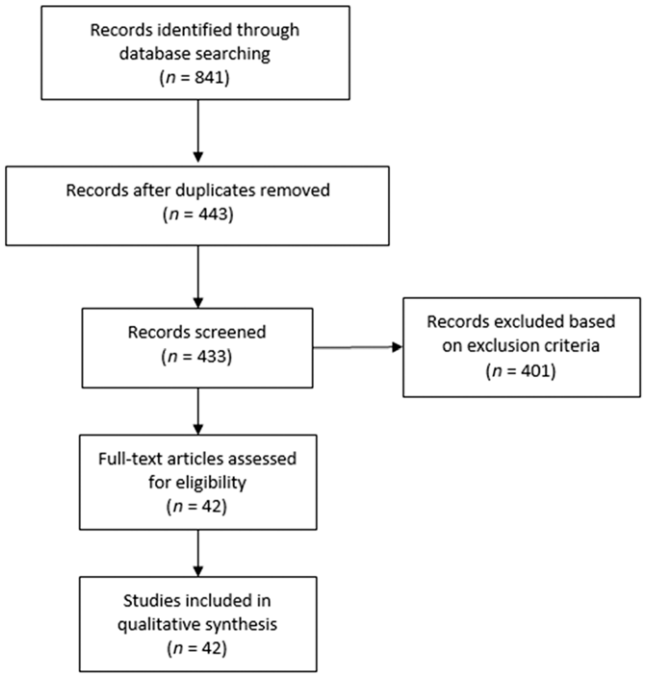

Figure 1
Flowchart of the study

diversity in the fungal flora in psoriatic skin lesions, compared to healthy skin was reported by Paulino *et al*. [25] and Amaya *et al*. [26]. No differences in the abundance of specific species was observed. Controversially, a significant dichotomy between the relative abundances of specific *Malassezia* species between healthy skin, and psoriatic skin lesions was found by Takemoto *et al*. [32]. Similar inconsistencies in findings were also observed in those studies assessing the microbiota [28–31, 34].

Hidradenitis suppurativa

To date, only two studies have been published that investigated the skin microbiome in HS (Table 1) [35, 36]. Both studies report a significant dysbiosis in HS lesional skin with more abundance of anaerobic genera. Five lesional microbiome types were identified of which type 1 (*Corynebacterium* species) and type IV (*Porphyromonas* and *Peptoniphilus* species) were most prevalent [35]. *Porphyromonas* was also found as predominantly abundant on lesional skin by Guet-Revillet *et al*. [36], together with *Prevotella* species. In addition, clinical severity significantly correlated with *Fusobacterium* and *Parvimonas* species variation in this study.

Ulcus cruris

The role of the skin microbiome in UC was explored in 10 different studies, Table 1 [37–46]. Current research into UC microbiome, comprises larger, longitudinal studies, compared to those in PV and HS. The skin mycobiota of diabetic foot ulcers was longitudinally assessed and was observed to be highly heterogeneous over time and between subjects while the diversity increased upon antibiotic treatment [45]. There have been similar efforts to reveal correlations between patient metadata, treatment and/or clinical outcomes and the cutaneous microbiome in studies investigating the microbiota in UC [38, 42–44, 46]. Overall, the most common found genus in these studies was *Staphylococcus*, with *Staphylococcus aureus* the most common species. Ulcer closing in diabetic patients was found to be positively correlated with higher microbial diversity and relative abundance of *Proteobacteria*, while a relative abundance of *Staphylococcus* was correlated negatively in a study by Gardner *et al*. [42]. Although *Staphylococcus* was consistently reported to be the most common genus, inconsistencies exist regarding other genus that are important in CU.

Seborrheic dermatitis/Pityriasis capitis

Four case–control studies investigated the microbiome in SD patients [47–50], Table 1. In general, *Malassezia* spp. were found to be more abundant on dandruff scalp compared to healthy scalp [47, 48, 50]. In addition to the mycobiota, a dysbiosis in *Staphylococcus* and *Propionibacterium* spp. was described in microbiota analysis [48, 50]. One of the four studies did not find a general association between *Malassezia* spp. and SD but did find a higher abundance of *M. globate* in severe SD patients [49].

Acne vulgaris

Five studies investigated the skin microbiome in patients with AV, Table 1 [51–55]. Three (3) were case–control studies and two (2) were small single-centre, controlled studies, of whom one was a double-blind, randomized-controlled trial. In general, all case–control studies demonstrated similarly an increased microbial abundance of *Propionibacterium acnes* in the skin microbiome of patients with AV, compared to healthy [51–53]. In addition, an association between a specific *P. acnes* strains and acne affected skin, and healthy skin respectively was demonstrated [51, 52]. Acne improved and *Propionibacterium* abundance decreased after various treatments, together with an increase of microbial diversity in the two controlled studies. Moreover, a positive correlation between *Propionibacterium* abundance and acne severity grade was found [54, 55].

Atopic dermatitis

The skin microbiome in patients with AD was assessed in 11 studies, Table 1 [56–66]. A greater proportion of longitudinal studies and 2 completed randomized controlled trials were performed in AD patients. There is general consensus across studies that skin affected by AD exhibits decreased bacterial diversity, as a result of an increased abundance of *S. aureus* [60–64, 66]. In particular, AD flare ups were associated with an increased proportion of *Staphyloccocus* sequences, and *S. aureus* abundance correlated with disease severity [60]. In line with these results, microbial diversity in AD lesions was inversely correlated with overall eczema severity as observed by the EASI [63], with several further studies also reporting taxonomic normalization and increased bacterial diversity in AD lesional skin, following various treatments [60, 61, 63, 66].

Table 1

Summary table of the studies included in the review

Source First author, year [ref]	Disease	Study design No. of patients	Sample collection methods	Analysis	Key findings	Weaknesses	Level evidence
Paulino *et al.* 2006 [18]	PV	Case control 3 PV/5 HV	Sterile swabs Lesional and nonlesional skin Multiple sampling in one PV and 2 HV	18S rRNA 5.8S rDNA	· *Malassezia* mycobiota substantially different PV *vs*. HV	· Small cohort	Low
Amaya *et al.* 2007 [19]	PV	Case control 22 PV/36 AD/30 HV	OpSite® transparent adhesive dressings Lesional and nonlesional skin	5.8S rDNA	· *Malassezia* species detected in overall sites higher in PV and AD compared to HV	· Small cohort · PV patients on treatment · Limited analysis · Different skin site collection PV *vs*. AD and HV	Low
Paulino *et al.* 2008 [20]	PV	Case control 1 PV/1 HV	Sterile swabs Lesional and nonlesional skin Multiple time points	5.8S rDNA	· Mycobiota relatively stable over time. · No significant dichotomy between PV and HV.	· Small cohort · Limited analysis	Low
Gao *et al.* 2008 [21]	PV	Case control 6 PV/6 HV	Sterile swabs Lesional and nonlesional skin	16S rRNA V1-V9	· Firmucutes more abundant in lesional skin PV *vs*. nonlesional skin and HV. · Actinobacteria less abundant in lesional skin PV *vs*. nonlesional skin and HV.	· Small cohort · No serial sampling	Low
Fahlen *et al.* 2011 [22]	PV	Case control 10 PV/12 HV	2-mm skin punch biopsies	16S rRNA V3-V4	· Most common phyla in PV and HV: Firmicutis, Proteobacteria, Actinobacteria. · Staphylococci and Propionibacteria were less common in psoriatic lesions	· Small cohort · No serial sampling · Variation in skin sample sites	Low
Alekseyenko *et al.* 2013 [23]	PV	Case control & Prospective longitudinal cohort study CC: 54 PV/37 HV PC: 17 PV/15 HV	Sterile swabs Lesional and nonlesional skin HV matched sites Multiple sampling	16S rRNA V1-V3	· Most common phyla in PV and HV: Firmicutis, Proteobacteria, Actinobacteria. · Combined relative abundance of *Corynebacterium*, *Streptococcus* and *Staphylococcus* was increased in psoriatic skin, compared to unaffected skin and healthy control skin	· Some patients on active treatment · Mainly severe patients	Low to moderate
Statnikov *et al.* 2013 [24]	PV	Case control 54 PV/37 HV	Sterile swabs Lesional and nonlesional skin HV matched sites	16S rRNA V1-V3 and V3-V5	· Microbiome signatures could be used to diagnose psoriasis	· No serial sampling	Low to moderate
Takemoto *et al.* 2015 [25]	PV	Case control 12 PV/12 HV	PV: psoriatic scales by tweezer HV: OpSite® transparent adhesive dressings	26S rRNA D1 – D2	· Psoriatic lesions exhibited significantly greater diversity compared to HV · *Malassezia restricta* levels were significantly higher in psoriatic lesions, compared to healthy controls	· Small cohort · No serial sampling · Only male patients · Different sample method PV and HV	Low

(continues)

Table 1

(Continued)

Source First author, year [ref]	Disease	Study design No. of patients	Sample collection methods	Analysis	Key findings	Weaknesses	Level evidence
Salava *et al.* 2017 [26]	PV	Case control 13 PV	Sterile swabs Lesional and n onlesional skin	16S rRNA V1-V3	• No significant differences microbial diversity between lesional and nonlesional skin	• Small cohort • No serial sampling • Variation in skin sample sites	Low
Tett *et al.* 2017 [27]	PV	Case control 28 PV	Sterile swabs Lesional and nonlesional skin	WMS sequencing	• Plaques at the ear had a significant decrease in microbial diversity, and increase in *Staphylococcus* abundance • At species level, no differences between lesional and nonlesional skin were observed	• Small cohort • No serial sampling • Some patients on active treatment	Low
Ring *et al.* 2017 [28]	HS	Case control 30 HS 24 HV	Biopsies Lesional and nonlesional skin	16S rRNA V3-V4 18S rDNA V3-V4	• Microbiome in HS significantly different from HV in lesional and nonlesional skin • Five microbiome types identified • Lesional skin consisted predominantly of *Corynebacterium* species (type I) and *Peptoniphilus* species (type IV) • *Propionibacterium* showed a significant higher abundance in HV	• Small cohort • No serial sampling	Low
Guet-Revillet *et al.* 2017 [29]	HS	Prospective cohort 65 HS	Sterile swabs Lesional and nonlesional skin	16S rRNA V1-V2	• Lesional skin consisted predominantly of anaerobes (*Porphyromonas* and *Prevotella* species) • Clinical severity significantly associated with variations in lesional microbiota • Fusobacterium associated with severe HS	• Small cohort	Low
Dowd *et al.* 2008 [30]	UC	Prospective cohort 10 VLU/10 DFU/ 10 PU	Debridement samples	16S rRNA V4	• Major populations include of all wound include: *Staphylococcus*, *Pseudomonas*, *Peptoniphilus*, *Enterobacter*, *Strenotrophomonas*, *Finegoldia* and *Serratia* species • Each wound type different profile, dependent on oxygen tolerance of the bacterial population	• Small study • No serial sampling	Low
Price *et al.* 2009 [31]	UC	Prospective cohort 7 DFU/7 NU/3 VLU/3 PSU/4 OTH	Wound base curette Multiple time points	16S rRNA V3	• Fastidious anaerobic bacteria of the Clostridiales family XI were the most prevalent bacteria in wounds	• Small study • Sampling time point variable	Low

(continues)

Table 1

(Continued)

Source First author, year [ref]	Disease	Study design No. of patients	Sample collection methods	Analysis	Key findings	Weaknesses	Level evidence
					• Wound microbiota from antibiotic treated patients were significantly different from untreated patients • In diabetic patients, *Streptococcus* was more abundant	• Patients on wide variety of treatments	
Price *et al.* 2011 [32]	UC	Cross-sectional 4 DFU/3 NU/3 VLU/2 OTH	Wound base curette Multiple samples taken	16S rRNA V3-V4	• The 10 most common genera included *Staphylococcus*, *Pseudomonas*, *Streptococcus*, *Anaerococcus*, *Ralstonia*, *Morganella*, *Porphyromonas*, *Peptoniphilus*, *Janthinobacterium* and *Corynebacterium* • Samples from different sites within individual wounds shared similarities in bacterial community compositions • Samples taken from different wounds were less similar than those taken from different sites within the same wound	• Small cohort • Patients on active treatment • No serial sampling	Low
Rhoads *et al.* 2012 [33]	UC	Cross-sectional 4 DFU/3 NU/3 VLU/2 OTH	Wound base curette	16S rRNA V1-V3	• The ten most common genera included *Staphylococcus*, *Pseudomonas*, *Streptococcus*, *Anaerococcus*, *Ralstonia*, *Morganella*, *Porphyromonas*, *Peptoniphilus*, *Janthinobacterium* and *Corynebacterium* • Samples from different sites within individual wounds shared similarities in bacterial community compositions • Samples taken from different wounds were less similar than those taken from different sites within the same wound	• Small cohort • Patients on active treatment • No serial sampling	Low
Gjodsbol *et al.* 2012 [34]	UC	Comparative 46 VLU	Filter paper pad & punch biopsies	16S rRNA V1-V3	• *Staphylococcus aureus* most found species • Multiple sampling over time lead to identification of additional species • No difference in outcomes different sample techniques	• No controls	Low

(continues)

Table 1

(Continued)

Source First author, year [ref]	Disease	Study design No. of patients	Sample collection methods	Analysis	Key findings	Weaknesses	Level evidence
Gardner *et al.* 2013 [35]	UC	Cross-sectional 52 DFU	Sterile swabs	16S rRNA V1-V3	• The most abundant OTU was *Staphylococcus*, with *S. aureus* the most common species • Ulcer closing was positively correlated with number of species level OTUs, higher microbial diversity, relative abundance of Proteobacteria, and negatively correlated with relative abundance of *Staphylococcus* • Ulcer depth was negatively associated with *Staphylococcus* abundance and positively associated with anaerobic bacteria relative abundance	• No serial sampling • No controls	Low
Wolcott *et al.* 2016 [37]	UC	Cohort 2963 910 DFU/916 VLU/676 DU/370 PSU	Sharp debridement at surface wound bed	16S rRNA V1-V3	• Neither patient demographics (age, gender, race, diabetes status) nor wound type influenced the bacterial composition of the chronic wound microbiome • *Staphylococcus* and *Pseudomonas* comprise the most prevalent genera present in the microbiota of chronic wounds, with *S. aureus* and *S. epidermidis* the most predominant species • Chronic wounds are frequently colonized by communalistic and anaerobic bacteria, including coagulation-negative *Staphylococcus*, *Corynebacterium*, and *Propionibacterium* species	• Unclear whether patients were on treatment	Low to moderate
Smith *et al.* 2016 [36]	UC	Cohort 20 DFU	Sterile swabs	16S rRNA V4	• The most commonly detected bacteria in all ulcers were *Peptoniphilus*, *Anaerococcus* and *Corynebacterium* species • In new ulcers, the most commonly detected bacteria were the above and *Staphylococcus* species • The majority of OTUs residing in both new and recurrent ulcers (>67%) were mostly Gram-positive cocci (*Staphylococcus*, *Streptococcus*, *Anaerococcus*, *Peptoniphilus* and *Finegoldia* • Lower HbA1c values and shorter duration of diabetes correlated with higher diversity within the ulcer	• Small cohort • No serial sampling • No controls	Low

(continues)

Table 1

(Continued)

Source First author, year [ref]	Disease	Study design No. of patients	Sample collection methods	Analysis	Key findings	Weaknesses	Level evidence
Kalan *et al.* 2016 [38]	UC	Prospective longitudinal cohort 100 DFU	Sterile swabs Multiple time point sampling	ITS1 rRNA	· Fungal microbiome was highly heterogeneous over time and between subjects · Fungal diversity increased with antibiotic administration · The proportion of the phylum Ascomycota were significantly greater at the beginning of the study in wounds that took >8 weeks to heal	· No controls · Most patients on active treatment	Low to moderate
Loesche *et al.* 2017 [39]	UC	Prospective longitudinal cohort 100 DFU	Sterile swabs Multiple time point sampling	16S rRNA V1-V3	· The most abundant genus identified was *Staphylococcus*, followed by *Streptococcus*, *Corynebacterium* and *Anaerococcus* · The major OTU attributed to *Staphylococcus* was S. *aureus* · Ulcer microbiota was highly dynamic, with community type transitions occurring approximately every 3.52 weeks · Microbiota community instability was associated with faster healing and improved outcomes · Exposure to systemic antibiotics destabilize wound microbiota, rather than altering overall diversity or relative abundance of specific taxa	· No controls · Most patients on active treatment	Low to moderate
Kuk Park *et al.* 2012 [40]	SD/PC	Case control 4 PC 3 HV	Sterile swabs	26S rRNA D1-D2	· *P. meleagrinum* and *P. chrusogenum* detected on dandruff scalp · *Malassezia* spp. 2 times more abundant on dandruff scalp	· Small cohort · No serial sampling	Low
Clavaud *et al.* 2013. [41]	SD/PC	Case–control 29 PC 20 HV	Sterile swabs In 20 PC patients lesional and nonlesional sampling	16S 28S-ITS	· *M. restricta* major fungal species on scalp PC and HV · *M. restricta and s. epidermidis* significantly more abundant on PC scalp · *Propionibacterium acnes* significantly less abundant on PC scalp · *M. restricta/P. acnes* ratio significantly higher in PC scalp	· Small cohort · No serial sampling	Low
Soares *et al.* 2015 [42]	SD/PC	Case control 9 SD (5 mild, 4 severe) 5 HV	Sterile swabs Scalp, forehead chin, shoulder and interface samples	5.8S/ITS2 rDNA	· In general, no association between *Malassezia* mycobiota and SD was found	· Small cohort · No serial sampling	Low

(continues)

Table 1

(Continued)

Source First author, year [ref]	Disease	Study design No. of patients	Sample collection methods	Analysis	Key findings	Weaknesses	Level evidence
					• Higher *m. globosa* abundance was found in nonscalp lesions of severe SD patients		
Park *et al.* 2017 [43]	SD/PC	Case control 29 SD 28 PC 45 HV	Sterile swabs Scalp samples	16 s rRNA V4-V5 ITS1 rDNA	• Higher abundance of *Staphylococcus* sp. and *m. restricta*, and lower abundance of *Propionibacterium* associated with scalp disease	• No serial sampling	Low
Bek-Thomsen *et al.* 2008 [44]	AV	Case control 5 AV/3 HV	Cyanoacrylate biopsy AV acne lesion face HV nose area	16S rRNA V1-V9	• Acne skin higher diversity, *P. acnes* and *S. epidermidis* most common species	• Small cohort • Only moderate to severe patients • No serial sampling • No nonlesional patient sampling	Low
Fitz-Gibbon *et al.* 2013 [45]	AV	Case control 49 AV/52 HV	Bioré® Deep Cleansing Pore strips Nose area	16S rRNA V1-V9	• No difference relative abundance *P. acnes* AV in HV. • Association specific *P. acnes* strain and acne.	• Some patients on active treatment • No serial sampling • No nonlesional patient sampling	Low
Barnard *et al.* 2016 [46]	AV	Case control 38 AV/34 HV	Bioré® Deep Cleansing Pore strips Nose area	WMS sequencing	• Association specific *P. acnes* strain and acne.	• Some patients on active treatment • No serial sampling • No nonlesional patient sampling	Low
Dreno *et al.* 2017 [47]	AV	Single-center, randomized-controlled, double-blind Erythromycin 4% OR Dermatocosmetic 26 AV	Sterile swabs Lesional and nonlesional skin Multiple time points	16S rRNA V4	• Different microbiota profiles on different sites. • Erythromycin treatment reduced the number of Actinobacteria, and dermocosmetic reduced Actinobacteria and *Staphylococcus* spp.	• Small cohort • Multiple samples excluded due to insufficient bacterial material	Moderate
Kelhala *et al.* 2017 [48]	AV	Single-centre, controlled study isotretinoin 0.4–0.6 mg kg^{-1} or lymecycline 300 mg twice daily 17 isotretinoin 11 lymecycline 16 HV	Sterile swabs Predose and after 6 weeks Cheek, back and armpit	16S rRNA V1-V3	• Positive correlation *Propionibacterium* abundance and acne severity grade • Both treatments reduced clinical acne grades • *Propionibacterium* decreased in cheek samples after both treatments • *Propionibacterium* decreased in back samples	• Small cohort • No nonlesional patient sampling	Moderate

(continues)

Table 1

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Source First author, year [ref]	Disease	Study design No. of patients	Sample collection methods	Analysis	Key findings	Weaknesses	Level evidence
					after lymecycline, but not isotretinoin treatment • Diversity increased after treatment		
Sugita *et al.* 2004 [58]	AD	Case control 13 AD/12 HV	OpSite® transparent adhesive dressings Lesional skin HV matched sites	26S and 5S rRNA intergenic spacer region 1	• *M. restricta* colonizes both AD and HV	• Small cohort • No serial sampling • Limited analysis • Patients on active treatment	Low
Dekio *et al.* 2007 [49]	AD	Case control 13 AD/10 HV	Sterile swabs Forehead skin	16S rRNA	• In both AD and HV there was a high rate of *Streptococcus* species • In AD *Strenotrophomonas maltophilia* was significantly more common	• Small cohort • No serial sampling • Patients on active treatment	Low
Kaga *et al.* 2009 [50]	AD	Case control 56 AD/32 HV	OpSite® transparent adhesive dressings Lesional skin AD Face HV	26S and 5S rRNA intergenic spacer region 1	• In mild and moderate AD, *M. restricta* was predominant over *M. globose* • In patients with severe AD, proportions of *M. restricta* and *M. globose* were almost identical	• Limited analysis • No serial sampling • Variation in skin sample sites • Patients possibly on active treatment	Low to moderate
Yim *et al.* 2010 [51]	AD	Prospective cohort 60	Sterile swabs 5 body sites	26S	• There were no significant differences between positive *Malassezia* culture, *Malassezia* species, and severity of AD	• Limited analysis • Patients on emollient treatment	Low to moderate
Akaza *et al.* 2010 [52]	AD	Case control 67	Sterile swabs Lesional and nonlesional skin Face and trunk	26S	• For the total number of *Malassezia* species, there were no significant differences between lesional and nonlesional areas	• No serial sampling • Patients on active treatment	Low to moderate
Kong *et al.* 2012 [60]	AD	Prospective cohort 12 AD/11 HV	Sterile swabs Multiple time points Baseline, flare, post-flare	16S rRNA V1-V9	• Flare ups were associated with an increased proportion of *Staphylococcus* sequences, particularly *S. aureus*, and correlated with disease severity • Increases in *Streptococcus*, *Propionbacterium*, and *Corynebacterium* species were observed following therapy	• Small cohort • Only moderate to severe patients • Different treatments regimens during flare	Low to moderate
Seite *et al.* 2014 [54]	AD	Prospective cohort Emolliens treatment 46	Sterile swabs Lesional and nonlesional skin Multiple time points	16S rRNA V1-V2	• Affected skin harboured a greater relative abundance of *Staphylococcus*, and in particular	• Large time between first and second sample • Only moderate patients	Low to moderate

(continues)

Table 1

(Continued)

Source First author, year [ref]	Disease	Study design No. of patients	Sample collection methods	Analysis	Key findings	Weaknesses	Level evidence
					S. epidermis, compared to healthy skin • Responders had increased microbial diversity and decrease in *Staphylococcus* species		
Chng *et al.* 2016 [55]	AD	Case control 19 medical history AD/15 HV/5 positive skin prick	Tape stripping anti-cubital fossa	16S rRNA V3-V6 WMS	• Nonflare, baseline skin microbiome signatures enriched for *Streptococcus* and *Gemella* in AD prone skin versus normal skin • Increased percentage of *S. aureus* carriers noted in AD cohort over control subjects	• Small cohort • No serial sampling • No lesional samples	Low
Gonzalez *et al.* 2016 [56]	AD	Randomized, placebo-controlled, single-blinded Topical steroid or Topical steroid + dilute bleach bath 21 AD/14 HV	Sterile swabs Lesional and nonlesional skin Multiple time points	16S rRNA V4	• Affected skin harboured a greater relative abundance of *S. aureus* • Microbial diversity at all lesional sites inversely correlated with overall EASI Index score • Taxonomic normalization occurred on lesional following treatments • Bacterial communities on lesional skin resemble nonlesional skin but remain distinct from healthy control skin	• Small study	Moderate
Seite *et al.* 2017 [57]	AD	Double-blind, Randomized, comparative Emollient A or Emollient B 53	Sterile swabs Lesional and nonlesional skin Multiple time points	16S rRNA V1-V2	• Significant increased levels of *Xanthomonas* genus in patients treated with emollient A • Levels of *Staphylococcus* genus increased between Day 1 and Day 28 in patients treated with emollient B	• Only moderate patients • No wash-out other treatments	Moderate
Kim *et al.* 2017 [59]	AD	Prospective cohort Wet dressings Topical steroids Antihistamines Antibiotics 27 AD 6 HV	Saline soaked gauzes	16S rRNA V1-V3	• Proportion of *Staphylococcus* significantly decreased after treatment • Diversity (Shannon Index) significantly increased after treatment	• Small study • Patients on wide variety of treatments • No nonlesional skin analysis	Low to moderate

AD, atopic dermatitis; AV, acne vulgaris; DFU, diabetic foot ulcer; HS, hidradenitis suppurativa; NU, neuropathic ulcer; OTH, other; OTU, operational taxonomic unit; PSU, post-surgical ulcer; PU, pressure ulcer; PV, psoriasis vulgaris; SD/PC, seborrhoeic dermatitis/pityriasis capitis; UC, ulcus cruris; VLU, venous leg ulcer

Table 2

Evaluation of the microbiome as clinical biomarker for each dermatological disease included in the review based on the criteria of a useful biomarker as defined by de Visser *et al.* [4]

Indication	Manuscripts (N)	Evidence level overall	Consistency	Therapeutic response	Dose–response relation	Relationship with disease	Recommendation for trial implementation
PV	10	Low	–	0	0	0	Negative, more evidence needed
HS	2	Low	+	0	0	+	Negative, more evidence needed
UC	10	Low	+	0	0	+	Negative, more evidence needed
SD	4	Low	–	0	0	+	Negative, more evidence needed
AV	5	Moderate	+	+	0	+	Positive
AD	11	Moderate	+	+	0	+	Positive

AD, atopic dermatitis; AV, acne vulgaris; HS, hidradenitis suppurativa; PV, psoriasis vulgaris; SD/PC, seborrhoeic dermatitis/pityriasis capitis; UC, ulcus cruris
Scoring system indicated as follows: +, studies in general report a positive outcome; 0, no studies available; –, studies in general report a negative outcome

Discussion

This systematic review provides an overview of the clinical studies that have investigated nonculture skin microbiome associated outcomes in AD, SD, AV, HS, PV and UC with the goal to explore its potential as biomarker in early phase clinical drug development with drug specific or disease specific application, as also referred to as type 3 or type 6 biomarker according to the classic definition of Danhof *et al.* [67].

Potential for microbiome as biomarker: AD and AV

From our analysis, there is some preliminary evidence that the skin microbiota may be a suitable disease specific biomarker for clinical trials of AD. This is due to the correlation between *Staphylococcus* abundance, microbiome diversity profile and disease severity that seems to exist in multiple trials, therewith complying with most of the criteria for a useful biomarker, Table 2 [4]. Objective data on the change of the microbiota may be valuable to support subjective AD efficacy scores in early phase clinical trials. However, it must be noted that the cause and effect relationship between skin microbiota dysbiosis and AD remains incompletely elucidated [68]. Currently, no evidence of benefit of antimicrobial interventions directed at reduction of *Staphylococcus* in patients with AD exists, only in secondarily impetiginized AD [69–71]. As multiple studies included in this review indicate that the skin microbiota within an individual patient varies over time [60, 61, 63, 64], there is need for longitudinal, frequent sampling and standard analysis studies. Nevertheless, it has proven its potential value and is recommended to apply in AD clinical trials, in particular when microbiota can serve also as drug-specific biomarker, i.e. for drugs with antimicrobial activity such as antimicrobial peptides that are currently in clinical trials for AD.

In AV, a strong, positive correlation between *Propionibacterium* and acne severity grade is reported [55]. Moreover, acne improved and *Propionibacterium* decreased after treatment, while the microbial diversity increased [54, 55]. Taking into account that a clear pathophysiological role of *P. acnes* exists and antimicrobial interventions are effective in AV [72, 73], the adoption of the skin microbiome as biomarker in acne drug development programmes is, although still in its infancy, suggested by our review (Table 2). Lesion clearance often takes a long time; therefore, the inclusion of microbiota is a valid option to monitor subclinical treatment effects and restoration of normal bacterial profile, i.e. rebiosis. Although a small uncertainty remains regarding the exact relationship between aberrations in the skin microbiome and acne [74], we conclude that there is definitely a potential for the microbiota as biomarker in clinical trials (Table 2). Another option would be to culture *P. acnes* instead of profiling the whole skin microbiota in clinical trials; however, with this approach a comprehensive overview and insight in the diversity will be missed.

PV, UC, hidradenitis and SD are lacking evidence

Although dysbiosis in psoriasis seems to exist in the micro- as well as the mycobiota, study findings are heterogeneous. Wide variability in study design, sampling methods, controllable factors and sequencing techniques between groups, in conjunction with small sample populations, could provide a possible explanation for this. Therefore, no clear recommendations can be made at this time. Future work focusing on serial sampling and longitudinal studying of skin microbiome populations it PV patients, may provide information on its potential applicability as biomarker, Table 2. From a clinical perspective, we know that antimicrobial and antifungal agents are not successful in the treatment of psoriasis, which suggests that it is less attractive to explore [75, 76]. However, since immune dysregulation is the key of psoriasis and recent investigations describe the extensive cross talk between the immune system and the microbiome, there may still be potential that should be explored [77]. For UC inconsistencies in study design, sampling methods and the heterogeneity of the disease group also limit the comparability of study findings. There appears to be a relationship between certain species, types of ulcers and ulcer duration [42, 46]. However, longitudinal studies with frequent standard sampling and

standard analysis procedures are necessary to make a recommendation. The finding of dysbiosis in HS skin microbiome mostly regarding anaerobic species that is mostly consistent in two different studies opens up opportunities for the skin microbiome as biomarker in this field, Table 2 [35, 36]. However, future studies will have to confirm this potential. In SD, three different sequencing methods were used in the three different studies [47, 49, 50]. This, together with the small sample populations, single time point sampling and poor study designs, might explain the heterogeneity in findings. Since there is a clear evidence that antifungal agents such as ketoconazole are effective in SD [78], it is recommended to further explore the skin microbiome's potential in this disease in future clinical trials.

Limitations and considerations

It is important to note that in all included studies, there was a high variability in study design and sampling methods between groups, which makes comparisons of specific findings difficult. Case–control studies (25/42, 60%) dominate research into the skin microbiome and skin disease. Patients are compared with healthy controls, capturing microbial profiles at a particular time, but have little predictive value in determining functionality, looking more at associations, and not causation. The small patient sample sizes across all studies may fail to account for interindividual differences within the study population. The poorly defined inclusion and exclusion criteria, with certain studies including actively treated patients in their sample population, could also confound potential findings. The standardization of controllable factors to reduce confounders was not well documented or maybe not performed in most of the included studies. As simple factors including but not limiting of age, ethnicity, environmental factors, soap use, hand-washing and the use of topical (antimicrobial) agents before sampling have been shown to alter microbial skin communities; documentation of these metadata is essential to draw valid conclusions [5, 8, 12, 60, 61, 79–81]. Multiple methods were used for skin microbiome sampling across the studies (i.e. swabs, biopsies, tape strips, wound curettes). Interestingly, all have been shown to exhibit a wide variation in biomass yield, microbial profile, human DNA contribution/contamination, sampling depth and discomfort level for the test subject [19, 62, 82–87]. In addition to the sampling method, the selection of sampling sites and sampling frequency are important factors that were not always considered in the included studies. Consistent sampling of the same anatomical area of skin in all individuals in study cohorts is essential in order to limit confounders, and allow for the accurate comparison of skin microbiome populations. Moreover, regarding analysis, only consistent use of specific primers to target specific hypervariable V regions, will allow for collation of data and comparison between multiple studies. It is clear that broadly used analysis methods in this review as shown in Table 1 count as a limitation for comparison. Taken all the above together, based on the level of evidence it is clear that our recommendations should be made with some caution. A standard approach for skin microbiome study design, collection, storage, processing and analysis as proposed by Kong *et al*. should be followed in future studies [17]. However, although the list of limitations and sometimes poor evidence might be assessed as a weak recommendation for the inclusion of cutaneous microbiome in dermatological trials, the recent finding that the gut microbiome partially explains the response/nonresponse to PD-1 immunotherapy in different cancer patients will foster research into microbiome in general [88, 89]. In addition, the relation between the gut microbiome in inflammatory bowel disease and response to infliximab was also recently highlighted [90]. In particular, when considering the reports about the role of the gut-skin axis that might influence many diseases including the here investigated skin disorders [91–93].

Conclusion

Only a small number of studies have consistently reported the cutaneous microbiome for skin diseases and chronic wounds. Our findings reveal that for two indications – AD and AV – there is preliminary evidence to support implementation of the skin microbiome as biomarker in early phase clinical trials. For PV, UC, SD and HS, there is insufficient evidence. More standardized microbiome-directed studies studying the effect of current treatments on the microbiome are needed to draw conclusions.

Competing Interests

There are no competing interests to declare.

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