The human skin microbiome in acne at the strain level

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Human skin microbiome

- Skin is the first line of defense against pathogens.
- The external surface of the human skin harbors 200-300 bacterial species. The number of bacterial cells of the skin is on the order of $10^{12}$.
- Different skin sites (dry, moist, or sebaceous) harbor distinct microbial communities.
- We are interested in studying the human skin microbiome in the pilosebaceous unit and its association with skin diseases.

http://www.acne.org/whatisacne.html
Acne vulgaris

- Acne is the most common skin disorder affecting approximately 50 million people in the States. More than 80% of the population suffers from acne at some point in their life.
- Acne is most prevalent during the middle-to-late teenage period.
- Acne can be extremely painful. It has significant morbidity and profoundly affects patients’ self-esteem, especially in adolescent population.
- Two main types of treatments: antibiotics and retinoids.
- Economically, it is estimated that U.S. consumers spend more than 1.2 billion dollars each year for the treatment of acne.
- The etiology of acne is still unclear. A bacterial factor has been believed to be one of the mechanisms contributing to acne.
Pilosebaceous unit has a tractable microbiome with a single dominant species.

The microcomedone is dominated by one bacterial species, *Propionibacterium acnes* (87%).
This system offers a unique advantage allowing in-depth analysis of a human microbiome at the strain level.
**Propionibacterium acnes**

- *P. acnes* is a Gram-positive, anaerobic bacillus that colonizes human skin.
- *P. acnes* has been implicated as a pathogenic factor in acne.
- In inflammatory acne, *P. acnes* plays a key role in eliciting a host inflammatory response that is thought to be important for the pathogenesis of the disease.
- Antibiotic therapy targeting *P. acnes* has been a mainstay treatment for more than 30 years.
16S rDNA sequencing of samples from acne patients and normal individuals

Samples from acne patients

Samples from normal individuals

Pooled normal skin samples by metagenomic shotgun sequencing

Relative abundance of species

- Propionibacterium acnes
- Propionibacterium granulosum
- Propionibacterium oral taxon 193
- Staphylococcus epidermis
- Staphylococcus capitis
- Prevotella oris
- Escherichia coli
- Other

16S rDNA sequencing of samples from acne patients and normal individuals
A few major ribotypes dominate the microbiome in pilosebaceous units.
Some of the major ribotypes are associated with acne or normal skin

<table>
<thead>
<tr>
<th>Ribotype</th>
<th>Nucleotide changes from RT1</th>
<th>Number of subjects</th>
<th>Number of clones</th>
<th>Percentage of clones from acne patients&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Percentage of clones from normal individuals&lt;sup&gt;b&lt;/sup&gt;</th>
<th>p-value&lt;sup&gt;c&lt;/sup&gt;</th>
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</thead>
<tbody>
<tr>
<td>RT1</td>
<td>-</td>
<td>90</td>
<td>5536</td>
<td>48%</td>
<td>52%</td>
<td>0.84</td>
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<tr>
<td>RT2</td>
<td>T854C</td>
<td>48</td>
<td>1213</td>
<td>51%</td>
<td>49%</td>
<td>0.36</td>
</tr>
<tr>
<td>RT3</td>
<td>T1007C</td>
<td>60</td>
<td>2104</td>
<td>40%</td>
<td>60%</td>
<td>0.092</td>
</tr>
<tr>
<td>RT4</td>
<td>G1058C, A1201C</td>
<td>23</td>
<td>275</td>
<td>84%</td>
<td>16%</td>
<td>0.049</td>
</tr>
<tr>
<td>RT5</td>
<td>G1058C</td>
<td>15</td>
<td>205</td>
<td>99%</td>
<td>1%</td>
<td>0.00050</td>
</tr>
<tr>
<td>RT6</td>
<td>T854C, C1336T</td>
<td>11</td>
<td>262</td>
<td>1%</td>
<td>99%</td>
<td>0.025</td>
</tr>
<tr>
<td>RT7</td>
<td>G529A</td>
<td>10</td>
<td>188</td>
<td>99%</td>
<td>1%</td>
<td>0.12</td>
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<tr>
<td>RT8</td>
<td>G1004A, T1007C</td>
<td>5</td>
<td>239</td>
<td>100%</td>
<td>0%</td>
<td>0.024</td>
</tr>
<tr>
<td>RT9</td>
<td>G1268A</td>
<td>4</td>
<td>68</td>
<td>99%</td>
<td>1%</td>
<td>0.29</td>
</tr>
<tr>
<td>RT10</td>
<td>T554C, G1058C</td>
<td>5</td>
<td>61</td>
<td>100%</td>
<td>0%</td>
<td>0.024</td>
</tr>
</tbody>
</table>
Five main microbiome types

Relative abundance of the top ten ribotypes

Acne (n=48) Normal (n=51)

Microbiome type I Microbiome type II Microbiome type III Microbiome type IV Microbiome type V Minor microbiome types
*P. acnes* population structures differ in acne and normal skin
Five main microbiome types were also observed in other cohort datasets.
RT6 is also abundant in the HMP cohort.

The relative abundance of the top ten ribotypes in different conditions is shown in the graph. The ribotypes are: RT10, RT9, RT8, RT7, RT6, RT5, RT4, RT3, RT2, RT1.

- Acne in this study
- Normal in this study
- HMP
- Grice et al. 2009
- Grice et al. 2009, HV4 removed

RT6 is highlighted in the graph.
The first *P. acnes* genome was published in 2004 (Bruggemann H *et al*.). The genome sequence of KPA171202 is 2.6Mb encoding 2,333 ORFs.

To understand the strain differences at the genome level, we isolated over 1,000 *P. acnes* strains from over 200 samples. 67 strains covering RT1, RT2, RT3, RT4, RT5, RT6 and RT8 were selected for sequencing to high quality genome drafts. 12 genomes were complete and finished.

There are additional 13 *P. acnes* genomes publically available.
Distinct lineages of *P. acnes* strains associated with acne or normal skin.
Pair-wise distance among the strains

RT4 and RT5 strains appear to be clonal.
RT2 and RT6 strains harbor CRISPR/cas

*E. coli* K-12 W3110

![Diagram of *E. coli* K-12 W3110 CRISPR/cas system]

*P. acnes* isolates with ribotypes 2 and 6

![Diagram of *P. acnes* isolates with ribotypes 2 and 6 CRISPR/cas system]
Closely related strains share CRISPR spacers

Some of the spacers encode sequences matching to loci 2 and 3 in RT4 and RT5.
Conclusions

- *P. acnes* is the dominant species in the pilosebaceous unit on human skin.
- The relative abundances of *P. acnes* are similar in acne patients and normal individuals, however, the population structure at the strain level is significantly different between the two cohorts.
- We found that RT4, RT5 and RT8 strains were highly associated with acne, and RT6 was enriched in healthy skin.
- We identified potential genetic determinants of various *P. acnes* strains in association with acne or health.
- Acquired DNA sequences and bacterial immune elements may play roles in determining virulence properties of *P. acnes* strains and some could be novel targets for therapeutic interventions.
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