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What does it take to satisfy Koch's postulates two centuries later? Microbial genomics and *Propionibacteria acnes*

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Abstract

For two centuries, Koch's postulates have set the gold standard for establishing the microbiological etiology of infection and disease. Genomic sequencing now brings finer resolution to both bacterial strain variation and the host genetic state that may predispose to disease. In this issue of the *JID*, Fitz-Gibbons and colleagues present strain based resolution of *Propionibacterium acnes* and its association with the common teenage malady acne vulgaris. Here I examine how Koch's postulates were envisioned and incorporate this finer resolution of both host and microbial states.

Keywords

genomics; Koch's postulates; acne

Developed in the 19th century, Robert Koch's postulates are the four criteria designed to assess whether a microorganism causes a disease. As originally stated, the four criteria are: (1) The microorganism must be found in diseased but not healthy individuals; (2) The microorganism must be cultured from the diseased individual; (3) Inoculation of a healthy individual with the cultured microorganism must recapitulated the disease; and finally (4) The microorganism must be re-isolated from the inoculated, diseased individual and matched to the original microorganism. Koch's postulates have been critically important in establishing the criteria whereby the scientific community agrees that a microorganism causes a disease.

Even Koch had to modify or bend the strictest interpretation of the first postulate. Koch discovered asymptomatic carriers of *Vibrio cholera* and *Salmonella typhi*, yielding the important distinction between asymptomatic clinical colonization and infection. Thus the field of inquiry into the intricate host-pathogen relationship was born.

Now genomics adds greater resolution to Koch's postulates in the context of common disorders which are not classical infectious disease. Acne vulgaris is a prevalent chronic inflammatory disease of the pilo-sebaceous unit characterized by follicular plugging (Williams *et al.*, 2012). Underlying factors associated with acne lesions are: (1) increased

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sebum production; (2) inflammation; (3) altered follicular keratinization; and (4) overgrowth with *Propionibacterium acnes*. However, over the years it has been difficult to ascertain which of these four factors initiates, promotes or is merely associated with disease state. In particular, to what extent does *P. acnes* contribute to the development of acne? The mainstay therapies of inflammatory acne include topical and systemic antibiotics, suggesting a microbial contribution. However, these common therapies often show incomplete response, and recurrence is frequent (Leyden, 2001). As well, systemic acne treatments sometimes possess untolerated side effects, suggesting that more targeted therapeutics would be beneficial.

P. acnes is a commensal Gram-positive anaerobe which is part of the microbial community of most sebaceous sites on normal healthy adults (Grice *et al.*, 2009).

But does overgrowth of *P. acnes* trigger skin inflammation? In this issue of the *JID*, Li, Craft and colleagues examine the strain level diversity of *P. acnes* of acne patients and healthy individuals by sequencing both direct clinical samples and *P. acnes* full genomes(Fitz-Gibbon *et al.*, 2013). Their rationale for pursuing this species level *P. acnes* resolution is based on classic epidemiologic studies that certain strains of micro-organisms are associated with different outcomes; e.g. methicillin-resistant *S. aureus*-USA300 or *Escherichia coli* 0157.

Fitz-Gibbons compared *P. acnes* strain diversity by direct sequencing of the signature bacterial 16S ribosomal RNA (rRNA) genes from ~50 acne patients and 50 normal controls. Skin microcomedone samples were obtained from the pilosebaceous units (pores) of the nose with Biore strips. Acne affectation was graded with the Global Acne Severity Scale, ranging from 0 (unaffected) to 5 (most severe), for subjects' face and nose. The acne cohort's severity ranged from 1 to 5 with an average of 2.1 and noses ranged from 0 to 2 with an average of 0.3. Acne and normal cohort had an average age of 22.2 and 29.6 respectively. Treatment information was collected from three-quarters of the total cohort with 9 reporting current treatment and 18 total reporting a history of treatment with antibiotics and/or retinoids.

Biore strips were placed individually in lysis buffer from which DNA was directly isolated. From each subject, ~400 16S rRNA clones were sequenced with 87% matching *P. acnes*. No difference was observed in the relative abundance of *P. acnes* between acne and normal cohorts. Fitz-Gibbons et al. assigned each 16S rRNA clone to a strain type based on single nucleotide variants and calculated the prevalence of each *P. acnes* strain in acne and normal cohorts. Of the ten most common strain types, the authors found that 3 were evenly distributed between the two cohorts, 6 were more often found in acne patients and 1 was associated with normal skin.

The full-length 16S rRNA gene is ~1500 base pairs and many of these strain types differed from each other by only 1 bp, so the authors next explored whether these small changes represent lineages at the full genome level. 66 *P. acnes* genomes, representing multiple isolates from many of the major strain types, were fully sequenced. Isolates from the same strain clustered together in the phylogenetic tree based on the full genome sequence,

suggesting that 16S rRNA sequences do reflect the evolutionary history of *P. acnes* lineages. The genomes of acne-enriched strains selectively harbor a plasmid and two chromosomal regions that contain genes possibly involved in virulence, adhesion to epithelial tissues or induction of human immune response.

Two letters to the editor by Alexeyev&Zoubloulis and Eady&Layton raise issues about the study design utilized by the authors' *P. acnes* research article. Both letters raise the concern that the nose is not an appropriate sampling site, as it is typically unaffected. While the acne cohort all had facial acne, only one-third had acne on the nose. Human studies have often found changes in gene expression or skin properties (such as barrier function) in unaffected skin sites, the authors might have gained greater specificity if acne lesions had been specifically sampled. These letters also raise issue with the technique deployed in this study, given that *P. acnes* resides deep within the anaerobic part of the hair follicle. Li & Craft's response provides further elucidation as to the typical sample obtained with a Biore strip and how the follicles were plucked off the strip with micro-forceps. These samples are thus considered 'pre-lesional' by Craft and Li.

Eady&Layton raises further concerns with the vague clinical history of the subjects utilized in this study, who are on average mid-twenties, or one decade past the typical age of acne onset. The oldest control subject is 80 years old with 8 control subjects aged 42 or older. For almost 20% of the total subjects, medication treatment histories were not given, but no matter since it's difficult for patients to accurately report antimicrobial usage in the last year, let alone one decade earlier. While a physician or trained study coordinator questioned every subject, a previous history of acne does not appear to be an exclusion for normal subjects. Current and previous medication usage is an unexplored confounder for these studies.

While Alexeyev&Zoubloulis and Eady&Layton's letters raise issues with study design, they are also intrigued by the potential to generate new testable hypotheses linking acne vulgaris to specific strains of *P. acnes*, which ultimately might lead to more targeted therapies. Identifying genes specifically encoded by more pathogenic *P. acnes* is an exciting new possibility from these genomic studies. It would be interesting to initiate a longitudinal study to examine the proportion of different ribotypes as teenagers progress through puberty and either do or do not develop acne. Samples could be obtained from affected and unaffected pores and analyses stratified for medication usage. This longitudinal survey could also examine if common therapies target specific strain types and whether efficacy is related to the initial community of *P. acnes* strains. However, even this level of resolution will only begin to satisfy Koch's postulates. The most challenging part will be moving from association to causality.

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