MICROBIOME

Prescription drugs obscure microbiome analyses

Patient populations should be stratified for medications when looking for gut microbial signatures of disease

By **Suzanne Devkota**

Although observations linking members of the gut microbiome to human disease have been plentiful, some are fraught with complex and confounding variables, emphasizing the need for vetting such associations with greater computational and mechanistic rigor. A recent study by Forslund *et al.* (*1*) adds another dimension for consideration by illustrating how medications may adversely affect the microbiome—an interaction often overlooked in post hoc analyses of disease-microbe relationships.

Focusing on type 2 diabetes, Forslund *et al.* used new and existing gut metagenomic data sets from 199 patients with type 2 diabetes and 554 nondiabetic controls from Danish (*2*), Swedish (*3*), and Chinese cohorts (*4*) to examine whether stratifying for metformin treatment, the most commonly prescribed antidiabetic drug, affected whether microbial signatures of disease were still apparent. The studies from which most of these data sets were derived could distinguish diabetic from nondiabetic patients based on such microbial signatures. However, Forslund *et al.* cite the lack of drug stratification as a potential confounder. When the patients with type 2 diabetes were stratified based on whether they were taking metformin or not, the microbial signatures between untreated patients with type 2 diabetes and nondiabetic controls were diminished, whereas the metformin-treated patients could be reliably predicted. These results suggest that either metformin treatment may be a bigger driver of the observed microbial differences than type 2 diabetes status itself, or that those individuals whose disease is adequately controlled by metformin alone define a unique subset of type 2 diabetes—an idea yet unexplored.

Indeed, there is increasing evidence that medications can profoundly affect microbial gene expression (*5*), and likewise, that the microbiota can transform drugs, affecting their bioavailability (*6*). In this context, however, metformin is intriguing because its mode of action—suppression of liver glucose production—is known, yet the mechanism by which this is carried out is unclear and open to testing. Studies in mice suggest microbial mediation of the drug's antihyperglycemic effects (*7*). However, the drug is excreted largely intact in the urine with no known metabolites, essentially excluding the possibility of transformation of the drug by the bacteria.

Although Forslund *et al.* do not examine the mechanism by which metformin appears to affect the microbiome, they do make a testable observation that untreated patients with type 2 diabetes have a decrease in beneficial butyrate-producing bacteria, which is reversed with metformin treatment. This is consistent with their findings that metformin increases the functional potential for short-chain fatty acid production, specifically butyrate and propionate. The authors, in turn, hypothesize that short-chain fatty acids alter intestinal glucose production, previously shown in rats (*8*), which may favorably affect liver glucose production and overall glycemic regulation. When controlling for gender, body mass index, and fasting blood glucose or insulin concentration, metformin treatment was further associated with increases in *Escherichia* spp. (except in the Chinese cohort) and a decrease in *Intestinibacter*. Although the relevance of this interplay is unclear, this may be a case of competitive exclusion whereby metformin is somehow creating a favorable environment for the *Escherichia*

F. Widjaja Foundation Inflammatory Bowel and Immunobiology Research Institute, Cedars-Sinai Medical Center, Los Angeles, CA, USA. E-mail: suzanne.devkota@cshs.org

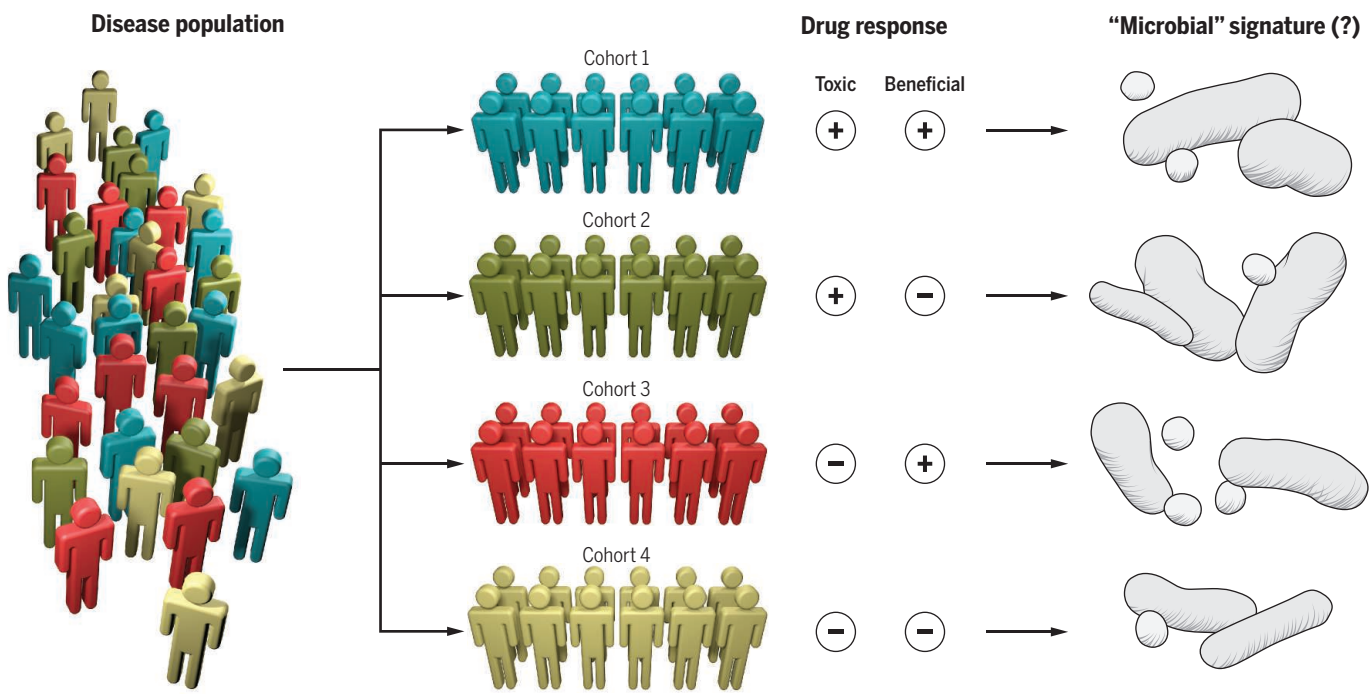

ILLUSTRATION: P. HUEY/*SCIENCE*

Microbial signatures. For a disease such as type 2 diabetes, patients should be stratified based on medications to reveal drug-microbe interactions.

spp. to thrive where *Intestinibacter* cannot, or by directly compromising the integrity of the microbe. Evidence for the latter can be found in a pharmaco-materials study whereby metformin attached to gold nanoparticles had the highest antibacterial and bactericidal activity against pathogens by compromising the cell wall when compared to other nonantibiotic drugs (*9*).

Interestingly, a cohort that was missing from the Forslund *et al.* study, and that would lend insight into the above observations, is the 30% of patients with type 2 diabetes who cannot tolerate metformin due to gastrointestinal distress. This common side effect often prevents patients from taking what is otherwise a relatively safe, effective, and inexpensive drug. Understanding whether these individuals have a distinct microbiome signature of their own that predisposes them to the unpleasant side effects creates an opportunity to alter the offending microbes through diet or other means, such that metformin becomes a viable treatment option. Within the existing metformin-treated cohort in the Forslund *et al.* study, the authors suggest that microbial genes encoding virulence factors and involved in gas production are enriched. However, the associations are unclear, as the implication for inclusion in this cohort is that these individuals are tolerant to the drug. Furthermore, metformin is often prescribed in combination with another antidiabetic drug, and it remains unclear whether the stratification also accounted for combination therapy.

Overall, Forslund *et al.* make a strong case for the importance of stratifying for any ubiquitously prescribed drug in a disease of interest when looking for microbial signatures (see the figure). For prospective studies, exclusion criteria aim to identify such confounding factors and exclude them in the first place. However, for most diseases, it is rare to find enough patients who have not undergone treatment to sufficiently power a study; nor is it ethical to take a patient off a drug that is controlling a disease for the purpose of a study. Therefore, we are left with the less than ideal option of keeping careful patient records and stratifying post hoc. However, what this study truly underscores is the need for more investigation into drug-microbiome interactions and the mechanisms therein. ■

REFERENCES

1. K. Forslund *et al.*, *Nature* **528**, 262 (2015).
2. E. Le Chatlier *et al.*, *Nature* **500**, 541 (2013).
3. F. H. Karlsson *et al.*, *Nature* **498**, 99 (2013).
4. J. Qin *et al.*, *Nature* **490**, 55 (2013).
5. C. F. Maurice *et al.*, *Cell* **152**, 39 (2013).
6. H. J. Haiser *et al.*, *Science* **341**, 295 (2013).
7. N. Shin *et al.*, *Gut* **63**, 727 (2014).
8. M. Croset *et al.*, *Diabetes* **50**, 740 (2001).
9. Y. Zhao *et al.*, *J. Am. Chem. Soc.* **135**, 12940 (2013).

10.1126/science.aaf1353

CANCER

Tracking the origins of tumorigenesis

A zebrafish model allows visualization of embryonic reprogramming during melanoma initiation

By **Soufiane Boumahdi**[1] *and* **Cédric Blanpain**[1,2]

Cancer arises through mutations that transform normal cells into cells that proliferate in an uncontrolled manner, form a tumor, invade the underlying tissue, and then metastasize to distant organs (*1*). Although the genetic events required to induce tumor formation are relatively well known (*2*), the additional early downstream molecular events that are required to reprogram normal cells into cancer cells are still poorly understood. On page 464 of this issue, Kaufman *et al.* report the development of an elegant transgenic reporter system that allows the early steps of tumor initiation to be tracked in situ. They find that oncogene-expressing melanocytes are reprogrammed into neural crest–like progenitors before progressing into invasive tumors (*3*).

Melanomas arise from the transformation of melanocytes, pigment-producing cells, which are derived from neural crest progenitors (NCPs) during embryonic development (*4*). Melanoma formation is associated with mutations in *BRAF*, N-*RAS*, and other oncogenes or tumor suppressor genes (*5*). In zebrafish, melanocytes are responsible for the pigmented stripes located on the scales of the fish. Transgenic overexpression in fish melanocytes of a mutated form of *BRAF* [with the mutation $Val^{600} \rightarrow$ Glu (V600E), the most frequent driver mutation in human melanoma] induced the formation of benign nevi, mole-like features; the concomitant deletion of *p53* promoted the progression of these nevi into malignant melanomas (*6*). Even though all melanocytes expressed the $BRAF^{V600E}$ oncogene and were deficient for *p53*, very few eventually formed melanomas, indicating that other mechanisms besides $BRAF^{V600E}$ and *p53* loss of function are needed for tumor initiation.

To better elucidate these mechanisms, Kaufman *et al.* generated transgenic zebrafish to visualize and characterize the early steps of melanoma formation in situ. They engineered fish expressing a crestin-GFP (green fluorescent protein) reporter gene, which faithfully recapitulates crestin expression during embryogenesis and in melanomas (*7*). Crestin-GFP is invariably expressed, prior to the malignant transition, in all lesions that will eventually progress into invasive tumors; this suggests that crestin-GFP marks a point of no return during tumorigenesis and represents one of the earliest molecular states associated with tumor initiation. The survival and propagation of crestin-GFP–expressing cells after transplantation in the scales of $BRAF^{V600E}/p53^{-/-}$ fish further supports the idea that these early crestin-GFP^{+} patches are already tumorigenic. The reexpression of markers of embryonic NCP cells during melanoma initiation supports the notion that oncogene-expressing cells progressing into invasive tumors are reprogrammed into a state that resembles their embryonic progenitor counterpart. The embryonic reprogramming of adult stem cells during tumor initiation was previously reported during initiation of basal cell carcinoma, the most frequent cancer in humans (*8*).

> ***"...crestin-GFP marks a point of no return during tumorigenesis..."***

The authors identified a 296–base pair minimal promoter/enhancer element that regulates crestin-GFP transgene expression during embryonic development and melanoma formation. This element contains binding sites for multiple transcription factors, including Sox10, Pax3, Mitf, and Tfap2, that regulate NCP specification and differentiation (*9*). Mutations in these transcription factor binding sites decreased the specificity of crestin-GFP transgene expression during embryogenesis, supporting the notion that Sox10 together with Mitf and Tfap2 control crestin-GFP expression during melanocyte development. It will be important to assess

[1]*Université Libre de Bruxelles, Institut de Recherche Interdisciplinaire en Biologie Humaine et Moléculaire, Brussels B-1070, Belgium.* [2]*WELBIO, Université Libre de Bruxelles, Brussels B-1070, Belgium. E-mail: cedric.blanpain@ulb.ac.be*