# Quantifying the effect of gut microbiome variation on interpersonal differences in drug metabolism

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## INTRODUCTION

Individuals vary widely in their response to medical drugs, and growing evidence implicates the gut microbiome, together with host genetics, in this interpersonal variation. Alarmingly, bacterial drug metabolism can modify drugs' activity and toxicity, causing altered drug response and adverse effects. However, the extent, the mechanisms and the implications for human health of microbiome drug modulation remain understudied and largely neglected in preclinical drug research.

In our previous work we found that two thirds of 271 tested drugs were metabolised by individual gut bacteria. This raised the question of how these results translate to complex microbial gut communities, and to what extent the interpersonal differences in microbiome composition impact microbial drug metabolism. We hypothesise that interpersonal differences in microbiome drug metabolism play a key role in inter-individual variation in drug metabolism and response. In this study, we set up an experimental in vitro model to systematically assess the occurrence and extent of variation of drug metabolism of gut communities derived from humans and the most common preclinical animal models.

## METHODOLOGY

Ex-vivo cultures of 90 gut microbial communities (60 from humans and 30 from animals representing six different species routinely used in drug discovery research) were anaerobically incubated with 271 orally administered clinical drugs, broadly representing the chemical drug space and different indication areas. Microbiota composition was obtained by metagenomic shotgun sequencing of all communities before drug exposure to comprehensively characterise the taxonomic compositions and gene contents of the different gut communities. For the analysis of drug metabolism, longitudinal data were collected at nine time points over 24 hours of drug incubation. Liquid chromatography-coupled mass spectrometry (LC-MS) analysis was performed to quantify drug degradation over time upon exposure to microbial gut communities. Further, to link the observed drug-metabolising capacity of each microbial community to their respective composition, we performed correlation analyses between drug degradation velocity and relative taxonomic

abundance of specific taxonomic groups or genes that were previously identified to mediate microbial drug metabolism.

## RESULTS

Metagenomic analysis revealed 435 bacterial species in the assayed microbial gut communities, including species previously reported to have drug-metabolising activity. Taxonomically the communities showed highly variable composition, with the human gut communities showing the highest interindividual variability and being clearly distinct from that of animal species. We collected more than 20000 microbiota-drug interaction time profiles, with each community metabolising between 15 and 77 drugs with a total of 205 drugs being metabolised. Notably, drugs that were previously identified to be metabolised by individual bacteria were also metabolised by communities. Although the majority of drugs were either metabolised by all or none of the microbial communities, many drugs showed significant interindividual variation in metabolism, that we linked to community composition via linear regression modelling. For example, the velocity of Famciclovir and Roxatidine metabolism could be explained by the relative abundance of Bacteroidetes species (SCC=-0.470, p-val=1.71E-4, SCC=-0.509, p-val=4.97E-5, respectively) supporting the hypothesis of community-specific drug degradation ability.

## DISCUSSION

In this study, we developed a systematic approach to experimentally link microbial community composition, its genetic potential, and drug metabolism. Combining metagenomics and metabolomics analyses, we set up a high-throughput screen to quantify microbiome-derived drug metabolism in human and animal gut communities. We found that the velocity of drug metabolism differed between different microbial communities in a drug-specific manner, illustrating that both the molecular features of drug compounds and the microbiome composition matter for drug metabolism. The difference in microbiome drug metabolism observed between individuals may suggest implications in interpersonal differences in drug response. Further, the fact that the laboratory animal models only poorly reflect the human microbiome diversity raises the question of how the variability in human microbiome drug metabolism is reflected in preclinical animal studies. Overall, our results strengthen the hypothesis that gut microbiota represents a neglected factor in the interaction between humans and drugs, and they suggest thatmicrobiota drug metabolism plays a role in interpersonal variation in drug response.