

Trends

Advances in research and medical practices have made significant inroads towards the treatment of diseases at the single patient level. This paradigm, precision medicine, holds the promise of reducing adverse effects, improving preventative care, and reducing costs by tailoring individual treatment based on highly detailed diagnostics.

Humans harbor trillions of microbes, termed the microbiome, which is now being appreciated as being a hugely substantial facet of health. Immune, metabolic, neurological, and other processes impact and are impacted by the microbiome.

The microbiome not only is a significant factor in health, but it is one that can be both readily assayed through DNA sequencing and directly modified by various targeted interventions. Therefore, the currently genetic information dominated field of precision medicine would be greatly enhanced by the introduction of the microbiome.

1 **Introducing the Microbiome into Precision Medicine**

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11

12 **Abstract**

13 Understanding how individual people respond to medical therapy is a key facet of
14 improving the odd-ratio that interventions will have a positive impact. Reducing the non-
15 responder rate for an intervention or reducing complications associated with a particular
16 treatment or surgery is the next stage of medical advance. The Precision Medicine
17 Initiative, launched in January 2015, set the stage for enhanced collaboration between
18 researchers and medical professionals to develop next-generation techniques to aid
19 patient treatment and recovery, and increased the opportunities for impactful preemptive
20 care. The microbiome plays a crucial role in health and disease, as it influences
21 endocrinology, physiology and even neurology, altering the outcome of many different
22 disease states, and it augments drug responses and tolerance. We review the implications

23 of the microbiome on precision health initiatives and highlight excellent examples,
24 whereby precision microbiome health has been implemented.
25

26 **Introduction to Precision Medicine**

27 The sequencing of the human genome [1] in 2001 fostered advances in both our
28 understanding of the genomic basis of disease and in the DNA sequencing technologies
29 required to bring the results of this understanding to patients. This is often referred to as
30 precision genomic medicine, which utilizes a patient’s individual genome to inform
31 treatment and care, based on known genomic markers for disease [2]. The broader,
32 inclusive field of precision medicine couples a person’s treatment with what is known
33 about their population, life style, and medical history, by matching clinical data and
34 genetic biomarkers. Since the genome is sometimes conceptualized as the core of human
35 individuality, at least in terms of disease, the broader field of precision medicine is often
36 conflated with genomic medicine. Precision medicine, however, includes aspects
37 downstream from the genome, including gene expression and protein expression as well
38 as metabolic markers. Nonetheless, genomic information is the most commonly used and
39 has had great successes [3]. Cancer treatment in particular has been revolutionized by
40 genomic medicine [4], which exemplifies that despite difficulties in implementing
41 precision medicine, it is a deeply important development. In particular, achieving the
42 goals of precision medicine, including diagnosing disease more accurately and reducing
43 the relative risk of treatments, side effects, and non-responses to medications, will
44 revolutionize both treatment courses — ideally at the single patient level [5] — and the
45 structuring of medical care and costs, moving towards cheaper, preventative focused
46 medicine.

47

48 **The Microbiome as a Precision Medicine Frontier**

49 In this review we focus on a more recent but in many ways analogous development, that
50 of introducing the microbiome into precision medicine. The human microbiome is the
51 “the ecological community of commensal, symbiotic, and pathogenic microorganisms
52 that literally share our body space” [6]. These microorganisms, mainly bacteria, fungi,
53 archaea, and viruses in the gastrointestinal tract, are slightly more abundant than the
54 human cells in the body, leading some to classify them as an newly discovered organ [7].
55 It is important to note however that the microbiome is compositionally and
56 spatiotemporally far more fluid and mutable than human cells and organs. Therefore, the
57 microbial “organ” may be better described as a “cloud” of genetic information accessory
58 to the stable human genome [8]. Certainly, the influences of the microbiome on our
59 physiology are significant and multitudinous, affecting immunology [9], neurology
60 [10,11], endocrinology [12], and, importantly for precision medicine, disease states and
61 clinical outcomes. Because microbiome science is a nascent but quickly developing field,
62 additional important functions of the microbiome are likely still to be discovered. These
63 discoveries are driven by similar sequencing technology as that which has enabled
64 personal genomics, and this technology is decreasing rapidly in price [13], so much so
65 that personal microbiome sequencing is already available to the consumer (e.g. American
66 Gut - americangut.org; uBiome - ubiome.com). Furthermore, the well-developed analysis
67 and statistical techniques of genomic medicine have commonalities with microbiome
68 analysis. Since microbiome states are highly individual even between co-raised identical
69 twins [14], but can be rapidly changed [15] (unlike genetics), there is a profound
70 opportunity for individualized treatments. However, the microbiome, like any ecosystem
71 is also profoundly complex, and so the goals of precision microbial medicine require

72 considerably more research before they are appropriately realized [16]. Nonetheless, the
73 microbiome, as we shall exemplify here, is primed and ready for precision medicine, and
74 therefore the clinical application of this new therapeutic area is on the immediate horizon.
75 Various complementary routes of assaying and modifying the microbiome have been
76 proposed and tentatively utilized towards this end; these will be laid out here in the
77 following text as well as diagrammatically (Figure 1).

78

79 **Review of Microbiome Analysis Techniques**

80 How then could microbiome precision medicine be implemented? Currently two
81 complementary analyses, both beginning with the extraction of microbial genomic DNA,
82 are standard in the field: 16S rRNA sequencing and shotgun metagenomics. The 16S
83 rRNA gene has both highly conserved regions, allowing for the usage of extremely
84 bacterially nonspecific primers, and “hypervariable” regions, where base pair differences
85 can often provide species level identification [17]. Thus, 16S rRNA amplicon sequencing
86 provides a robust tool for identification as well as classification and even discovery of
87 bacteria [18]. A typical 16S rRNA study utilizes the differences in observed communities
88 of bacteria between differing samples to obtain statistically significant correlations
89 between bacterial composition and sample description, for example to identify
90 differences in the gut microbiomes of children born to obese mothers [19]. These studies
91 have led to key insights into the human microbiome. While historically the majority of
92 biomedical research on bacteria has focused on eliminating pathogens, many bacteria as
93 well as communities of bacteria are important in both health and disease [6]. Though
94 identifying causative bacteria in disease states will be an important facet of precision

95 medicine, understanding the overall ecology of the microbiome may be equally or even
96 more vital.

97

98 Therefore, to go beyond bacterial identification and subsequent, limited patient
99 stratification, it will be essential to understand the functional potential of the microbiome.
100 Shotgun metagenomics enables the researcher to understand this function potential
101 through analysis of the complete genomic repertoire of the community, by sequencing
102 DNA extracted from that community, rather than relying on amplification of a marker
103 gene. Taxonomy can still be determined from signature genes (including 16S rRNA), but
104 it is also possible to assign phylogeny of the functional genes by comparing the DNA
105 sequence against a library of genomes from close relatives [20]. In addition,
106 metagenomics enables the assembly of genomes from organisms in the microbiome that
107 are resistant to culture, providing a higher resolution exploration of the taxa associated
108 with each person [21]. This enables us to determine the metabolic and signaling capacity
109 of each taxon, to determine how it will interact with the rest of the body [22]. This clearly
110 makes metagenomics of great interest for the development of precision medicine;
111 however, one must be aware of the challenges this technique presents. Metagenomic
112 studies are necessarily more expensive and computationally complex than 16S rRNA
113 based studies. Possible contamination from undesired DNA and biases of analyses
114 towards culturable organisms [23] further complicate matters. Ultimately metagenomics
115 is an extremely useful tool, but the application of this technology to precision medicine
116 will require a better understanding of the implications of these limitations, especially
117 when scaling up to treatments of large patient populations.

118

119 Notably, both 16S sequencing and shotgun metagenomics are currently somewhat blunt
120 tools, especially when describing the fluid nature of the microbiome. Evolution of
121 microorganisms, horizontal transfer of genes, and subtleties in the characterization into
122 types of microbiomes [24] problematize the microbiome snapshot style data often
123 acquired. As sequencing costs continue to decrease, however, scientists can sample more
124 densely in time to capture previously unobservable subtleties in microbial interactions
125 and utilize time series techniques to uncover dynamic ecological phenomena [25].
126 Additionally, the gut microbiome is known to be spatially inhomogeneous, in ways that
127 influence function and disease states [26]. This limitation too might be surpassed in the
128 near future, owing to emerging sampling techniques and protocols (e.g., laser
129 microdissection of colonic crypt mucus [27]).

130

131 **Avenues Towards Microbiome-Based Precision Therapies**

132 *Microbiome-xenobiotic interactions*

133 That gene polymorphisms can drive changes in drug metabolism has been known for
134 some time; it was noted as early as 1957 that atypical forms of serum cholinesterase led
135 to potentially fatal reactions to certain anesthetics [28]. This and other adverse drug
136 reactions are estimated to cost from 30 to 130 billion dollars in the USA annually [29,30]
137 and are a significant source of patient non-compliance and therapy failure [31]. Reducing
138 these adverse reactions is a primary goal of precision medicine. While some interactions
139 are idiosyncratic, a recent survey of adverse drug events observed that about 35% of
140 these events were drug-gene or drug-drug-gene interactions involving cytochrome P450

141 oxidase (CYP) variants [32]. CYPs are generally considered the body's innate and
142 primary general purpose drug metabolizers; they are involved in about 75% of total
143 human drug modification [33].

144

145 However, microbial metabolism in the gut is also a significant factor in
146 biotransformation, especially for low solubility, low permeability compounds [34].

147 Currently, more than 60 drugs have been identified to have microbiome interactions

148 according to the PharmacoMicrobiomics database [35], and given the vast number of

149 possible unique microbial metabolic transformations [36], many more interactions are

150 likely to be discovered compared with the apparently relatively limited number of human

151 genetic interactions. The plasticity of the microbiome may make these interactions

152 dynamic, necessitating precision medicine that is not only patient specific but temporarily

153 appropriate [37]. Importantly, the primary forms of xenobiotic metabolism are different

154 between human and bacterial cells: oxidation and conjugation dominate in the former

155 case, reduction and hydrolysis in the latter [34]. Metabolism of drugs is actually a key

156 component of many therapies; so-called "prodrugs" are essentially drugs that will be

157 metabolized into a pharmacologically active drug after consumption. Therefore,

158 production of active drug metabolites from prodrugs is sometimes dependent on the

159 microbiome, with the possibility to either improve or worsen outcomes [38]. This often

160 manifests as a modulation of bioavailability to the human, an important consideration for

161 prediction of appropriate dosing in precision medicine. Efficacy and side effects are also

162 altered directly by microbial metabolism. For example, acetaminophen toxicity shows

163 substantial variability within a given human population [39], and the microbiome has

164 been identified as playing a role in this variability. Members of the genus *Clostridium*, as
165 well as other bacteria can produce *p*-cresol, which competes as a substrate for SULT1A1
166 (a human liver enzyme) with acetaminophen [40]. A reduction in the breakdown of
167 acetaminophen by SULT1A1 causes a build-up of NAPQI, which leads to hepatotoxicity.
168 This general pattern of competition between bacterial metabolites and drugs for human
169 enzyme modification constitutes a major challenge in pharmacology [41]. Directly
170 harmful substances can also be formed by microbiota, as is the case in bacterial β -
171 Glucuronidase mediated diarrhea in response to an antitumor camptothecin derivative
172 [42]. Strikingly, in some cases even strain level differences can lead to altered
173 metabolism, such as inactivation of digoxin by a non-universal *E. lenta* gene. Digoxin has
174 a narrow therapeutic window, and thus a wrong dosage could lead to significant toxicity,
175 highlighting the need for further study of metagenomic diagnostics and insights to
176 adverse outcomes [43].

177

178 Furthermore, alternative mechanisms for xenobiotic-microbiome interaction including
179 immune [9,44,45] and endocrine [12] modulation by bacteria are known to exist,
180 complicating and enlarging the pool of possible drug-microbiome interactions. Lastly,
181 there are possible reciprocal relations: drugs may both be altered by the microbiome and
182 alter the microbiome. For example, antipsychotic medication has been shown to both
183 alter the microbiome and have microbiome-dependent side effects [46]. While this
184 greatly complicates endeavors to understand microbiota-xenobiotic interactions, it also
185 points towards a different microbiome driven approach to precision medicine: directly
186 targeting the microbiome for clinical results.

187

188 *Targeting the microbiome*

189 It is clear that medication is already utilized to have a direct effect on the microbiome;
190 one needs to look no further than antibiotics. While these drugs are utilized for the
191 eradication of pathogenic bacteria, they have widespread effects on the microbiome,
192 possibly leading to adverse outcomes. Secondary infections caused by antibiotics are well
193 known, most saliently *Clostridium difficile* [47], but it is often less appreciated that
194 antibiotics can have side effects on the human, for instance fluoroquinolone associated
195 cardiotoxic [48] and neuropsychiatric [49] reactions. Importantly, consequences of
196 antibiotic usage, such as reduction of inflammation, are possibly not only human off-
197 target drug effects, but also unintended consequences of microbial community disruption
198 [50]. Studies using mouse models suggest that stress induced increases in circulating
199 cytokines were abrogated by broad-spectrum antibiotic treatment [51]. Furthermore,
200 these types of interactions are not limited to drugs classified as antibiotics; many other
201 drugs have antibiotic and other microbial community structure and function modulating
202 properties that are beginning to be appreciated [52,53]. While many of these
203 perturbations to the microbiome are associated with poorer outcomes, some drugs may
204 derive some or all of their beneficial qualities from alteration of the microbiome, thus
205 they could be considered a form of discriminatory antibiotic.

206

207 A precision medicine therapy that leverages microbial community structural modulation
208 could have beneficial clinical impact. Certainly if pathogen-specific antibiotics were
209 developed, the odds ratio could be greatly increased compared to traditional antibiotics. A

210 clear approach is to design a species-specific enzyme inhibitor or other antimicrobial
211 molecules. For example, a *Streptococcus mutans*-targeted drug based on the fusion of a
212 species-specific targeting peptide domain with a wide-spectrum antimicrobial peptide
213 domain has already been developed [54]. However, the bacterial community was also
214 altered when using this peptide, despite its high specificity [55]. This is likely because the
215 environment of *Streptococcus mutans*, the oral microbiome, presents significant
216 structural and functional complexity [56]. It has been suggested that targeted antibiotics
217 may shift the microbiome into a healthier state, but of course there is also the potential
218 for negative ecological effects, although these may be less than for traditional antibiotics.

219

220 An intriguing approach that may largely avoid the problem of system scale changes in
221 microbial community structure, as well as that of increasing antimicrobial resistance, is to
222 non-lethally target specific enzymes in the bacteria. This has been realized at the multi-
223 species level [57] through targeted inhibition of bacterial tri-methyl amine (TMA)
224 formation by 3,3-Dimethyl-1-butanol (DMB, a structural analog of choline) ultimately
225 attenuating atherosclerosis in a high choline diet mouse model. Surprisingly, slight
226 alterations of bacterial composition were still observed, underscoring the extremely
227 dynamic nature of the microbiome. Nonetheless, this study points towards a microbiome-
228 based intervention for a specific (i.e., “Western”) diet-driven disease. In this case, a
229 single target approach is undesirable, as reduction of global TMA formation is the goal,
230 but given the availability of single isozyme inhibitors [58], precision, non-lethal drugs
231 likely could be developed. These furthermore have the potential to be minimally
232 bioavailable to the human, limiting side effects, and might be exploited not only to target

233 pathogens but also to reduce microbiota-drug interactions through selective elimination
234 of problem microbes.

235

236 A final approach for targeted antimicrobials has been successfully employed for
237 approximately 100 years, though not as popularly in the western world [59]. Phages were
238 independently discovered in France and England, though developed as a therapy first in
239 the former. Despite great successes in treatment, especially of cholera, commercialization
240 of phage therapy failed due to production problems and other complications and so was
241 subsequently ignored in the US and Europe after the development of antibiotics [60].
242 Scientists in the Soviet Union (especially Georgia) continued to develop phage therapy,
243 having been cut off from antibiotic advances due to World War II. Here it was effectively
244 used it to control outbreaks of gastrointestinal diseases and refined further during the
245 Cold War and afterwards [61]. The basic premise of this technique is that many bacterial
246 species, and maybe even each strain (sub-species), are predated upon by a unique phage
247 [62]. Phage target bacteria cell-membrane protein and sugar complexes that are unique to
248 each bacterial taxon. Therefore, by identifying the correct phage it should be possible to
249 precisely remove a specific bacterial species from an assemblage. This will enable
250 accurate restructuring of a microbiome so as to precisely augment the functional
251 properties of that consortium. In fact, recent evidence from the commercial sector
252 suggests that the same mechanisms employed by phages to target and penetrate bacterial
253 cells can be programmed into nano-particles that mimic these phage-properties to infect
254 and kill specific cells (Pers. Comm. Jeffrey Miller, UCLA). In this new future, we may
255 have ultimate control over the microbiome.

256

257 *Prebiotic treatments*

258 Conversely, instead of targeting the microbiome to reduce deleterious bacteria, one could
259 aim to increase the levels of beneficial bacteria or otherwise positively alter the structure
260 or function of the microbiome. Substances applied in this way are often referred to as
261 prebiotics. However, the types of prebiotics currently studied are limited in scope, usually
262 non-digestible fiber compounds that stimulate growth of *Bifidobacterium* and other taxa
263 to produce short chain fatty acids (SCFA) including butyrate and propionate [63].
264 Though this is promising as a broad treatments for several conditions [64], efforts for
265 precision medicine in this sphere will require the expansion of the scope of prebiotics.
266 Given that metagenomic and metabolomic advances continue to better characterize the
267 metabolic potential of the microbiome, especially across groups with vastly different
268 diets [65], prebiotic compounds that stimulate alternative beneficial bacteria towards
269 useful metabolic endpoints will be discovered [66].

270

271 More audaciously, one might aim at fine-tuning the interactions between microbiota of
272 the gut microbiome. The microbiome is a complex, human co-evolved ecosystem that
273 produces many bioactive compounds, often for intercellular communication [26]. These
274 compounds could be mined to find those which modulate the microbiome in a beneficial
275 way, thus unearthing novel prebiotics [67]. While microbial community disruption is the
276 consequence of both xenobiotic and microbiome targeted drug metabolism, these types of
277 prebiotics might provide a more gentle perturbation than possible with the former by
278 harnessing already existing biological pathways. This goal certainly seems distant, but as

279 dynamical systems approaches to studying the microbiome continue to develop, we may
280 find that treating certain dysbiotic states require perturbations of varying magnitudes or
281 delicate maintenance of the stability of the microbiome, especially in at-risk populations
282 [68].

283

284 *Precision probiotics*

285 Perhaps the most direct strategy for altering the microbiome is the usage of probiotics,
286 live microbes administered for health benefits. This idea has been employed since at least
287 1907 when Élie Metchnikoff hypothesized lactic acid producing bacteria could implant in
288 the gastrointestinal tract to enhance longevity [69]. Today the probiotic landscape is still
289 dominated by lactic acid bacteria, specifically genera *Lactobacillus*, though it is now
290 appreciated that their beneficial properties are not limited to the production of a single
291 metabolite and that other potential probiotic bacteria, perhaps isolated from healthy
292 individuals [70], could affect various outcomes through multifarious means [71]. This
293 opens the door to precision probiotic development since application of microorganisms is
294 highly specific with regards to both applied agent and effect. Devices now exist for
295 isolating microorganisms based on metabolic output [72], and work is being done to
296 identify probiotic bacteria that produce particular compounds of therapeutic potential
297 [73]. This may include compounds whose efficacies are contingent on route of
298 administration, for example those that are inactive orally. Furthermore, probiotics are
299 being bioengineered to expand their ranges and modes of actions as well as their
300 robustness and incorporation [74]. However, it is important to keep in mind that
301 interactions with diet, established microbiota, and genetics, are known to modulate

302 overall health outcomes if not specific effects and mechanisms of probiotics [71].
303 Therefore effective patient classification and stratification is required for best results.
304 Success of this program will require detailed insights into metagenomic potential and
305 ecological interactions of presumptive probiotic bacteria, making precision probiotic
306 development a task of considerable difficulty but one that has already seen demonstrable
307 results, for example in enhancing resistance to *Clostridium difficile* infection [75] and
308 suppressing hepatocellular carcinoma growth in mice [76].

309

310 **Regulation and Application**

311 Despite the therapeutic promise of the microbiome, its application to precision medicine
312 requires overcoming considerable hurdles. One may anticipate that failure to successfully
313 apply genomic medicine may lead to delays in the application of the microbiome as a
314 precision therapy. For example, the current legal and R&D model is not well suited for
315 development of genome-informed drugs [77]. Microbiome therapies likewise face
316 difficulties, especially owing to the wide breadth of treatment options, many of which
317 lack analogs in current medical practice. Furthermore, clinicians have been reticent to use
318 the results of genomic information — and thus likely future microbiome data — in
319 treatment due to both uncertainties on its importance and lack of understanding [78].
320 These problems are highlighted in the case of Plavix® (clopidogrel), whereby despite an
321 FDA box warning [79] indicating serious or fatal risk for those carrying certain
322 CYP2C19 variants, this drug is still routinely used on genetically incompatible patients
323 due to poor coverage by insurance and failure to clinically utilize genetic testing [80]. In
324 the case of the microbiome, fecal transplant treatment for *Clostridium difficile* colitis is

325 known to be highly effective especially in recurrent infection [81]; however, this
326 procedure still requires a licensed practitioner to have a protocol approved by their local
327 Institutional Review Board, and therefore each patient needs to be consented prior to
328 therapy. For a therapy with >90% success rate this is peculiar. However, it is because we
329 still lack the ability to characterize the microbial community of donor stool appropriately.
330 This means that we do not know the active components of the fecal transplant, and
331 therefore it is very difficult to regulate this using standard legislation under FDA
332 protocols. More importantly, we still don't fully understand the implications for
333 microbiome therapy on a large scale. While fecal transplants are becoming extremely
334 numerous with few legitimate side effects, it is still hard to predict the outcome across a
335 broad population. The same is true for genomic medicine, whereby the interaction of
336 genes with the environment is difficult to predict [82]. This requires enormous sample
337 populations for any investigation to be statistically significant [83]. Though the future is
338 bright for genomic medicine, particular issues currently impede efforts towards its
339 development.

340

341 Fortunately, some of the difficulties in genomic medicine research and deployment might
342 be lessened in precision microbiome medicine. Environmental-microbiome interactions
343 are potentially more easily studied because there is a more direct interaction between the
344 two, allowing for simpler identification of sample populations and achievement of
345 statistical power. With the correct experimental design, genetic variation can be
346 sufficiently decoupled from microbiome and environmental factors. In fact, studies of
347 this nature already exist, both on humans [84] and especially on mice, where genetics can

348 be well controlled [85]. This bottom-up approach can then be extended by genomic
349 studies which better account for confounding factors. Even where genetics is a significant
350 factor, such as in mental health disorders, incorporating the microbiome greatly increases
351 understanding and ultimately treatment of diseases [86]. Of course in disease states where
352 the effects of genetic variation are either entirely or nearly absent, the microbiome is a
353 great candidate for investigation. Conditions such as obesity [87] and inflammatory
354 bowel disease [88] can in subsets of patients be driven by dysbiosis, a chronic, systemic
355 maladaptation of the gut microbiome to the host. Unlike genomic medicine, there are
356 possibilities especially for these conditions to do research in an *in vitro* environment,
357 most excitingly in artificial gut paradigms [89]. Microbiome precision medicine also has
358 the opportunity to break free of present R&D and legal hurdles to precision medicine.
359 The regulation and marketing of these treatments will at least pose challenges for
360 traditional models [90,91], as evidenced by the FDA's current stance on probiotics [92],
361 which has led to faster product delivery to the public but also quality control and
362 effectiveness issues [93]. Prioritizing treatments will be an important aspect of achieving
363 R&D, FDA, and ultimately clinician support; unnecessary testing on low risk
364 communities and for low benefit interventions, will only hamper the development of
365 microbiome precision medicine.

366

367 **Notable Application: Medically Underserved Communities**

368 Given the above unique assets of the microbiome modality of precision medicine, a
369 promising potential area for its development is in low socio-economic status (SES) and
370 other under-served communities. Low SES is associated with reduced diversity in the gut

371 microbiome [94]. Numerous factors are also present especially in urban communities that
372 reduce immunoregulation, including reduced exposure to microbes in the natural
373 environment [95] and increased stress [96], and increase obesity prevalence and
374 dysbiosis, including increased density of fast-food restaurants [97] and lack of physical
375 activity [98]. This is likely interrelated with microbiome-associated diseases such as
376 asthma [99] and gastrointestinal symptoms [100]. The vast majority of genomic variants
377 discovered are either rare with large effects or common with small effects, unlike in this
378 situation where there is a possibility of appreciable effect size combined with biomarker
379 occurrence. Therefore, these at-risk communities present a potentially illuminating cohort
380 for microbiome.

381

382 Of course, great care must be taken to not draw inappropriate or invalid associations
383 between microbiome [101] (or genome [102]) variations and minority status. Lack of
384 cultural understanding and disparities in access to services have driven poor research
385 trends in the past and continue to be a deep issue in the development of precision
386 medicine. Access issues in particular have caused demonstrable problems; statistics on
387 epidermal growth factor receptor testing, for example, show associations of lower
388 educational attainment and income with reduced likelihood of testing [103], and studies
389 suggest health insurance coverage alone does not explain this general effect [104,105].
390 For precision medicine to succeed then, under-served populations must be both active
391 participants and beneficiaries of research. Microbiome research in particular could lead to
392 high impact clinical interventions for these communities, hopefully spurring its
393 development. It is both an opportunity and imperative for microbiome precision medicine

394 to address social epidemiological trends, but this is only possible through the combined
395 efforts of researchers, clinicians, the government, and perhaps most importantly the
396 people at large.

397

398 **Concluding Remarks**

399 Here we have presented a collection of potential avenues towards introducing the
400 microbiome into precision medicine. Though it is difficult to know if and when these
401 techniques will ultimately make it to the clinic (see Outstanding Questions), there is
402 substantial evidence that microbiome-based medicine holds great future potential to
403 improve odds-ratios, reduce side effects, stratify patients, and precisely treat previously
404 difficult or untreatable conditions. Ultimately, the microbiome must become an integral
405 part of precision medicine as a whole, since so much of human functioning and
406 metabolism is dependent upon it. If this is to happen in the near future, as it hopefully
407 should, we must better understand the microbiome and its interactions with the human
408 and the environment via a concerted effort and conversation between researchers,
409 clinicians, patients, the government, and most importantly, the broader community.

410

411 **Figure 1**

412 A schematic of methods in precision microbiome medicine and their possible interplay:
413 a) As an example, certain microbes, here represented in red, metabolize the compound
414 cycasin to produce a carcinogenic compound methylazoxymethanol (MAM) [106]. This
415 functional potential of the microbe might be discovered through metagenomic
416 sequencing. b) If targeted removal of the red microorganism — identified in a patient via

417 16S sequencing — was desired, without harming commensal bacteria, represented in
418 shades of blue, three approaches (green arrows) might be utilized. Direct removal of the
419 deleterious microorganism through targeted antibiotics ideally would not affect
420 commensal bacteria. Probiotic treatment introduces new beneficial microorganisms while
421 prebiotic treatment favors the growth of existing beneficial microorganisms. Note that
422 prebiotic and probiotic treatments do not directly remove the targeted microorganism, but
423 in certain cases may shift the gut ecology such that it does not thrive [107]. In all three
424 cases, the specific circumstances may affect which treatment is best employed and what
425 residual outcomes there are on the microbiome.

426

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663

Outstanding Questions

What is the relative significance of specific microbial actors versus whole microbiome ecology in disease states, and how will drugging specific bacteria affect ecological succession following this perturbation? How will this depend on the milieu in which a species is situated (e.g., presence of different taxa performing a similar ecological role)? Additionally, what roles might phages, fungi, viruses play?

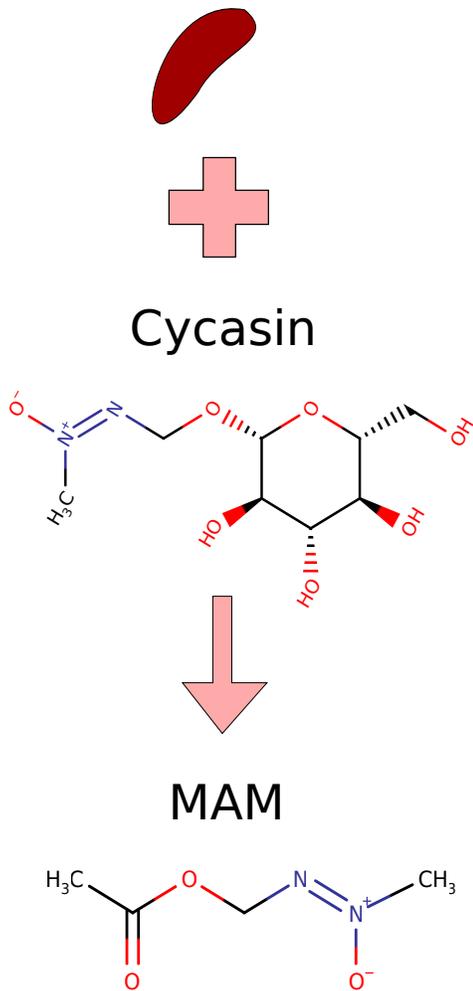
How closely coupled are genetics and the microbiome, and how can these fields be integrated into a unified practice of precision medicine?

Which microbiome-driven disease states can be successfully cured? Which instead require prophylactic or palliative, noncurative therapy?

What is the best way to move precision microbiome medicine results out into the clinic? What changes in regulatory, governmental as well as research and development processes will need to occur for this to happen?

How will the needs of different groups be best addressed across diets, lifestyles, and environments? What interventions will ultimately require social change rather than medical therapy, and what will the interplay between these fields be?

Figure 1
(A) *Metagenomic insight*



(B) *16S assay and diagnosis*

