OPEN ACCESS Freely available online

PLOS | MEDICINE

Research in Translation

A Physicians' Wish List for the Clinical Application of Intestinal Metagenomics

Ingeborg Klymiuk[1], Christoph Högenauer[2], Bettina Halwachs[3,4], Gerhard G. Thallinger[3,4], W. Florian Fricke[5], Christoph Steininger[6]*

1 Core Facility Molecular Biology, Center for Medical Research, Medical University of Graz, Graz, Austria, **2** Department of Gastroenterology and Hepatology, Medical University of Graz, Graz, Austria, **3** Institute for Genomics and Bioinformatics, Graz University of Technology, Graz, Austria, **4** Core Facility Bioinformatics, Austrian Centre of Industrial Biotechnology, Graz, Austria, **5** Department of Microbiology and Immunology, Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, Maryland, United States of America, **6** Division of Infectious Diseases, Department of Medicine I, Medical University of Vienna, Vienna, Austria

Introduction

The intestine is one of the most diverse and complex bacterial habitats of the human body, harboring ~1,000 bacterial phylotypes [1]. Recent studies have associated the human intestinal microbiome (i.e., the collective genomes of all intestinal microbial habitants [2]) with health and disease states, suggesting that metagenomic analysis of the intestinal microbiome could be exploited as a novel diagnostic, prophylactic, or therapeutic strategy in multiple medical specialties. For example, the identification and quantification of opportunistic pathogens in the intestinal microbiome may facilitate risk stratification in immunocompromised patients, such as in critically ill, HIV-infected or immunosuppressed (e.g., organ transplant recipients or individuals with autoimmune disease) patients. Also, the correction of intestinal dysbiosis, the pathologic imbalance of the gut microbiota, may inhibit the development and/or delay the progression of autoimmune diseases [3,4], metabolic disorders [5], and cancer [6]. The propagation of a healthy intestinal microbiota has even been shown to reduce toxicity and increase effectiveness of cancer therapies in rats [7]. In addition, standard analysis of the human intestinal microbiome in patients may enable the rapid identification of novel emerging infectious pathogens in fecal specimens, for example, in the case of an outbreak of Shiga-toxigenic *Escherichia coli* [8].

Our understanding of the human intestinal microbiome in health and disease has been revolutionized by the development of next generation sequencing and its application to metagenomics, which is the term generally used to summarize culture-independent technologies that allow the characterization of a microbiome [2]. These methods allow for the largely unbiased characterization of complex microbial communities at high resolution, including the detection of novel and uncultivable bacteria, viruses, archaea, and small eukaryotic organisms, even in compartments previously considered to be sterile, such as the urinary bladder [9]. The European MetaHIT project (http://www.metahit.eu) and the US National Institutes of Health Human Microbiome Project (http://www.hmpdacc.org) have

Summary Points

- Multiple infectious, autoimmune, metabolic, and neoplastic diseases have been associated with changes in the intestinal microbiome, although a cause–effect relationship is often difficult to establish.
- Here we discuss the problems, applications, and visionary requirements for the integration of microbiome analysis into clinical routine diagnostics.
- Metagenomics is increasingly used for the culture-independent and largely unbiased characterization of complex bacterial habitats at high resolution. The versatility and decreasing costs of metagenomics make this technology an interesting tool for clinical diagnostics.
- Methodological shortcomings still impede the application of metagenomics in clinical diagnostics.
- Integration of metagenomics into clinical medicine requires accepted and validated strategies for (1) translation into clinical action items; (2) sample collection, preparation, and testing; and (3) data analysis and interpretation. We highlight tasks that are of high priority from a clinical perspective for the useful medical application of metagenomics.

Research in Translation discusses health interventions in the context of translation from basic to clinical research, or from clinical evidence to practice.

Citation: Klymiuk I, Högenauer C, Halwachs B, Thallinger GG, Fricke WF, et al. (2014) A Physicians' Wish List for the Clinical Application of Intestinal Metagenomics. PLoS Med 11(4): e1001627. doi:10.1371/journal.pmed.1001627

Published April 15, 2014

Funding: The Austrian Centre of Industrial Biotechnology (ACIB) contribution was supported by FFG, BMWFJ, BMVIT, ZIT GmbH, Zukunftsstiftung Tirol and Land Steiermark within the Austrian COMET program FFG Grant 824186 (BH, GGT) and the EU COST Action SeqAhead, EC Grant BM1006 (GGT). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing Interests: The authors have declared that no competing interests exist.

Abbreviations: FMT, fecal microbiota transplantation; IBD, inflammatory bowel disease.

* E-mail: christoph.steininger@meduniwien.ac.at

Provenance: Not commissioned; externally peer reviewed

set new standards for the in-depth metagenomic characterization of the healthy human microbiota (microorganisms living inside and on humans) from different body habitats [2].

Optimizing patient outcome according to metagenomic information depends on the quality of the available information, options for translation of this information into clinical action, and the effectiveness of communication. Translation of metagenomic knowledge into clinical practice is impeded by several limitations. For example, vast amounts of information are generated by metagenomics, which has to be assorted, interpreted, and communicated to clinicians in a comprehensible format. Most clinical studies have focused on characterizing the human microbiota by its taxonomic composition using 16S rRNA–based bacterial surveys, although similar biological functions may be exerted by unrelated taxa [10]. Establishing a cause–effect relationship or using microbiome profiles as surrogate markers for diseases is accordingly difficult.

Priorities for the Application of Metagenomics in Clinical Medicine

Strategies still remain to be defined for (1) translation into clinical action items with impact on patient outcome; (2) sample collection, preparation, and testing; and (3) data analysis, interpretation, and communication. Here, we highlight the tasks that are of high priority from a clinical perspective for the useful application of metagenomics in clinical medicine.

Priority 1: Integration of Metagenomic Information with Other Clinical and Laboratory Sources of Evidence for Translation into Targeted Therapy

Metagenomic information has been associated with specific disorders in several studies. For example, clinical observations have long suggested that the intestinal microbiome plays a critical role in the pathogenesis of inflammatory bowel disease (IBD) (Crohn disease and ulcerative colitis): (1) inflammation in Crohn disease disappears if the involved bowel segment is excluded from the fecal stream and recurs after re-anastomosis with reexposure to intestinal contents [11]; (2) IBD responds at least partially to antimicrobials [12] and some probiotics (live bacteria or yeast preparations) [13]; (3) some studies have shown for IBD a decreased bacterial diversity and a shift from anti-inflammatory commensals to pro-inflammatory pathogens (dysbiosis)—particularly to an overrepresentation of proteobacteria and to a reduction in *Faecalibacterium prausnitzii* and other beneficial butyrate-producing bacteria [14–16].

While current evidence strongly suggests that the pathogenesis of IBD could be linked to the intestinal microbiota, important clinical questions remain unanswered. So far, study results analyzing microbiome changes in IBD patients were not controlled for potential confounders such as mucosal inflammation per se [17,18], accelerated intestinal transit due to diarrhea [19], or medications used for IBD treatment, for example, antibiotics and immunosuppressants [20,21]. In addition, evidence from animal models still has to be confirmed in human clinical medicine, such as the anti-inflammatory properties of *F. prausnitzii* in chronic intestinal inflammation [22]. Results from clinical studies are sometimes incongruous—initial studies of patients with ulcerative colitis showed a marked benefit from fecal microbiota transplantation (FMT) [23], but other small studies could not confirm this observation [24]. Another study showed that FMT could correct the proposed features of the dysbiotic intestinal microbiota in IBD, such as the increased abundance of proteobacteria, but did not result in significant clinical improvement [24].

Hence, metagenomics approaches have to fulfill several clinical prerequisites to have a significant impact on diagnostic, prophylactic, and therapeutic strategies. A cause–effect relationship between a defined disorder and intestinal microbiome profile has to be established beyond doubt. A clear distinction between intestinal microbiome profiles of disorders (e.g., IBD versus other causes of intestinal inflammation) on the basis of metagenomic information would greatly facilitate diagnostic strategies. Identification of significant confounders of metagenomic information (inflammation, concomitant therapy, diet, etc.) may also help in devising novel prophylactic strategies. Well-directed strategies for the targeted therapy of disorders of the intestinal microbiome have to be developed, and existing ones optimized (e.g., selection of FMT donors according to a target microbiome). For this purpose, longitudinal studies with well-defined intervention and control groups as well as adequate follow-up periods are warranted. Metagenomic information on longitudinal changes in the intestinal microbiome needs to be combined with other clinical and laboratory sources of evidence for translation into targeted therapies.

Priority 2: Standardization of Diagnostic Procedures in Sample Collection, Preparation, and Testing

Accurate sample collection, preparation, and analysis are of paramount importance for the characterization of the intestinal microbiome in health and disease. Collection of stool samples; collection of gastric, intestinal, or biliary fluid; and endoscopic mucosal biopsies are routine clinical procedures. Next generation sequencing already allows characterization of the microbial composition of a sample (e.g., by 16S rRNA gene region analysis) and of its genetic and functional potential (reviewed in [25,26]).

Nevertheless, the choice of sample, sampling procedure, and analytical workflow greatly influences the results and thus the clinical utility of metagenomic characterization. Microbiota compositions fluctuate in response to dietary and sanitary habits, age, genotype, sex, ethnicity, and use of antibiotic and other medications [27–29]. Sample contamination from other anatomic regions (e.g., from oropharynx to stomach) is difficult to avoid with currently available endoscopic tools [30]. The clinically most significant anatomic locations in relation to a specific intestinal disorder still have to be defined (e.g., fecal sample versus endoscopic biopsy, or sampling of lesions versus surrounding, unaffected mucosa in IBD). Finally, differences in sample preparation, DNA isolation, metagenomic approaches, number of reads analyzed, and sequencing instrument used have a large impact on the final results [27].

Standardization of workflows in metagenomic studies is therefore urgently needed. Sampling methods have to be developed to avoid carryover contaminations. Standards must to be adapted and optimized to specific human cohorts and diseases for a meaningful interpretation of metagenomic information.

Priority 3: Automation of Data Analysis, Interpretation, and Communication

Analysis and statistical interpretation of the data in a reproducible form are also vital for the translation of

metagenomics information into clinical action items [31]. Basically, sequence reads from the sampled DNA are clustered into operational taxonomic units, which are taxonomically classified and compiled into a list of relative operational taxonomic unit abundances for each sample (reviewed in [32]). Next, the whole-community composition can be statistically evaluated and categorized for clinical purposes according to function, prevalence, absence, or alternation of particular bacterial groups. These groups of interest can range from broad taxonomic classes to specific bacterial families or species, such as the two phyla Firmicutes and Bacteroidetes, whose ratio has relevance to obesity [33]; members of the phylum Proteobacteria, whose abundance has been associated with intestinal disease states such as IBD [18]; Clostridia species that induce anti-inflammatory regulatory T-cells [34]; or tumor-inducing *Fusobacterium nucleatum* [35].

Currently, the introduction of metagenomic tools into clinical practice is facing major technical as well as biological obstacles: (1) long analysis times, (2) evolving definitions of reference microbiota, (3) missing standards of analysis methods, algorithms, and databases, (4) lack of well-defined physiological ranges, and (5) missing evidence for cause–effect relationships.

From a technical perspective, a maximum level of automation would facilitate the digest of metagenomic data into clinically meaningful information. Analysis speed is highly dependent on the number of collectively analyzed samples, and the methods and tools used. Filtering and quality improvement steps may require several days, even on medium-sized computing clusters. Hence, rapid data analysis needs a reference microbiome as a reliable standard with which to compare individual samples, reduction of analysis complexity, and, ultimately, integration of analysis algorithms and desktop sequencers into a single package. Furthermore, for meaningful interpretation and communication, results of statistical evaluations should be generated and digested into clinically relevant bits automatically in the same sequencing unit, and communicated as an analysis report to the physician within a few hours. A crucial biological point is the definition of physiological ranges of gut microbiota parameters, which are highly variable between ethnic groups, geographic locations, and different diets [36]. For the definition of reference values, representative samples from the local healthy population have to be analyzed for the relative abundance of taxonomic groups or ratios between groups, combined with relevant clinical data (see the Human Microbiome Project and the American Food Project [http://humanfoodproject.com]). This information would also provide the basis for establishing cause–effect relationships. Finally, reference values have to be updated continuously and integrated into analysis algorithms for effective translation of evolving insight into intestinal microbiota into clinical practice.

Outlook

The establishment of characteristic and thoroughly validated signatures of the intestinal microbiome allows the development of new prophylactic, therapeutic, and prognostic strategies for beneficial and targeted modification of the patient's intestinal microbiome. Most metagenomic tools required for addressing these important questions are already available, standard operating tools are under development (see the Human Microbiome Project), and insight into the human microbiome is evolving rapidly (Box 1). Modern, high-resolution, and high-throughput analysis of complex bacterial communities in clinical samples has the potential to revolutionize clinical practice. As a prerequisite, target conditions must be specified, conclusively linked with characteristic signatures of the intestinal microbiome, and thoroughly validated. In addition, sample collection, preparation, testing, analysis, and result interpretation must be standardized and widely automated, and costs per sample and turnaround times significantly reduced. The integration of metagenomic analysis into clinical diagnostics will very likely open whole new avenues to the treatment of intestinal as well as extra-intestinal diseases.

Author Contributions

Conceived and designed the experiments: IK CH BH GGT WFF CS. Analyzed the data: IK CH BH GGT WFF CS. Wrote the first draft of the manuscript: IK CH BH GGT WFF CS. Contributed to the writing of the manuscript: IK CH BH GGT WFF CS. ICMJE criteria for authorship read and met: IK CH BH GGT WFF CS. Agree with manuscript results and conclusions: IK CH BH GGT WFF CS.

Box 1. Five Key Papers on the Translation of Metagenomics into Clinical Practice

1. **Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, et al. (2010) A human gut microbial gene catalogue established by metagenomic sequencing. Nature 464: 59–65.** This study reports a large-scale approach to characterizing the functionality of the intestinal microbiota by cataloging human gut microbial genes, which is a prerequisite for defining health and disease states in terms of the microbiome.
2. **Human Microbiome Project Consortium (2012) Structure, function and diversity of the healthy human microbiome. Nature 486: 207–214.** This project is a trendsetting approach to establishing comprehensive metagenomic datasets of (healthy) body habitats as reference datasets and to lay the foundation for the translation of metagenomic research into diagnostic applications.
3. **Kump PK, Gröchenig HP, Lackner S, Trajanoski S, Reicht G, et al. (2013) Alteration of intestinal dysbiosis by fecal microbiota transplantation does not induce remission in patients with chronic active ulcerative colitis. Inflamm Bowel Dis 19: 2155–2165.** This was one of the first attempts not only to use FMT but also to characterize the procedure and the outcome by metagenomics.
4. **Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. Nature 444: 1022–1023.** This study links the metagenomics pattern of the human intestinal microbiome to a clinical disorder and is therefore of importance for therapeutic approaches.
5. **Navas-Molina JA, Peralta-Sánchez JM, González A, McMurdie PJ, Vázquez-Baeza Y, et al. (2013) Advancing our understanding of the human microbiome using QIIME. Methods Enzymol 531: 371–444.** This study describes one of the common interactive analysis tools for microbiome analysis currently used by many researchers, which might be used in the future for standardizing data analysis.

References

1. Human Microbiome Project Consortium (2012) A framework for human microbiome research. Nature 486: 215–221.
2. Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, et al. (2007) The human microbiome project. Nature 449: 804–810.
3. Sellitto M, Bai G, Serena G, Fricke WF, Sturgeon C, et al. (2012) Proof of concept of microbiome-metabolome analysis and delayed gluten exposure on celiac disease autoimmunity in genetically at-risk infants. PLoS ONE 7: e33387.
4. Sjoberg V, Sandstrom O, Hedberg M, Hammarstrom S, Hernell O, et al. (2013) Intestinal T-cell responses in celiac disease—impact of celiac disease associated bacteria. PLoS ONE 8: e53414.
5. Henao-Mejia J, Elinav E, Jin C, Hao L, Mehal WZ, et al. (2012) Inflammasome-mediated dysbiosis regulates progression of NAFLD and obesity. Nature 482: 179–185.
6. Sobhani I, Tap J, Roudot-Thoraval F, Roperch JP, Letulle S, et al. (2011) Microbial dysbiosis in colorectal cancer (CRC) patients. PLoS ONE 6: e16393.
7. Lin XB, Dieleman LA, Ketabi A, Bibova I, Sawyer MB, et al. (2012) Irinotecan (CPT-11) chemotherapy alters intestinal microbiota in tumour bearing rats. PLoS ONE 7: e39764.
8. Loman NJ, Constantinidou C, Christner M, Rohde H, Chan JZ, et al. (2013) A culture-independent sequence-based metagenomics approach to the investigation of an outbreak of Shiga-toxigenic Escherichia coli O104:H4. JAMA 309: 1502–1510.
9. Wolfe AJ, Toh E, Shibata N, Rong R, Kenton K, et al. (2012) Evidence of uncultivated bacteria in the adult female bladder. J Clin Microbiol 50: 1376–1383.
10. Eckburg PB, Bik EM, Bernstein CN, Purdom E, Dethlefsen L, et al. (2005) Diversity of the human intestinal microbial flora. Science 308: 1635–1638.
11. Rutgeerts P, Goboes K, Peeters M, Hiele M, Penninckx F, et al. (1991) Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. Lancet 338: 771–774.
12. Khan KJ, Dubinsky MC, Ford AC, Ullman TA, Talley NJ, et al. (2011) Efficacy of immunosuppressive therapy for inflammatory bowel disease: a systematic review and meta-analysis. Am J Gastroenterol 106: 630–642.
13. Kruis W, Fric P, Pokrotnieks J, Lukas M, Fixa B, et al. (2004) Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. Gut 53: 1617–1623.
14. Lepage P, Hasler R, Spehlmann ME, Rehman A, Zvirbliene A, et al. (2011) Twin study indicates loss of interaction between microbiota and mucosa of patients with ulcerative colitis. Gastroenterology 141: 227–236.
15. Manichanh C, Borruel N, Casellas F, Guarner F (2012) The gut microbiota in IBD. Nat Rev Gastroenterol Hepatol 9: 599–608.
16. Sokol H, Pigneur B, Watterlot L, Lakhdari O, Bermudez-Humaran LG, et al. (2008) Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A 105: 16731–16736.
17. Gill N, Ferreira RB, Antunes LC, Willing BP, Sekirov I, et al. (2012) Neutrophil elastase alters the murine gut microbiota resulting in enhanced Salmonella colonization. PLoS ONE 7: e49646.
18. Lupp C, Robertson ML, Wickham ME, Sekirov I, Champion OL, et al. (2007) Host-mediated inflammation disrupts the intestinal microbiota and promotes the overgrowth of Enterobacteriaceae. Cell Host Microbe 2: 119–129.
19. Gorkiewicz G, Thallinger GG, Trajanoski S, Lackner S, Stocker G, et al. (2013) Alterations in the colonic microbiota in response to osmotic diarrhea. PLoS ONE 8: e55817.
20. Dethlefsen L, Relman DA (2011) Incomplete recovery and individualized responses of the human distal gut microbiota to repeated antibiotic perturbation. Proc Natl Acad Sci U S A 108 (Suppl 1): 4554–4561.
21. Morgan XC, Tickle TL, Sokol H, Gevers D, Devaney KL, et al. (2012) Dysfunction of the intestinal microbiome in inflammatory bowel disease and treatment. Genome Biol 13: R79.
22. Carlsson AH, Yakymenko O, Olivier I, Hakansson F, Postma E, et al. (2013) Faecalibacterium prausnitzii supernatant improves intestinal barrier function in mice DSS colitis. Scand J Gastroenterol 48: 1136–1144.
23. de Vrieze J (2013) Medical research. The promise of poop. Science 341: 954–957.
24. Kump PK, Grochenig HP, Lackner S, Trajanoski S, Reicht G, et al. (2013) Alteration of intestinal dysbiosis by fecal microbiota transplantation does not induce remission in patients with chronic active ulcerative colitis. Inflamm Bowel Dis 19: 2155–2165.
25. Loman NJ, Constantinidou C, Chan JZ, Halachev M, Sergeant M, et al. (2012) High-throughput bacterial genome sequencing: an embarrassment of choice, a world of opportunity. Nat Rev Microbiol 10: 599–606.
26. Soergel DA, Dey N, Knight R, Brenner SE (2012) Selection of primers for optimal taxonomic classification of environmental 16S rRNA gene sequences. ISME J 6: 1440–1444.
27. Muegge BD, Kuczynski J, Knights D, Clemente JC, Gonzalez A, et al. (2011) Diet drives convergence in gut microbiome functions across mammalian phylogeny and within humans. Science 332: 970–974.
28. Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, et al. (2013) Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science 341: 1241214.
29. Turnbaugh PJ, Quince C, Faith JJ, McHardy AC, Yatsunenko T, et al. (2010) Organismal, genetic, and transcriptional variation in the deeply sequenced gut microbiomes of identical twins. Proc Natl Acad Sci U S A 107: 7503–7508.
30. Yang I, Nell S, Suerbaum S (2013) Survival in hostile territory: the microbiota of the stomach. FEMS Microbiol Rev 37: 736–761.
31. Fricke WF, Rasko DA (2014) Bacterial genome sequencing in the clinic: bioinformatic challenges and solutions. Nat Rev Genet 15: 49–55.
32. Kuczynski J, Lauber CL, Walters WA, Parfrey LW, Clemente JC, et al. (2011) Experimental and analytical tools for studying the human microbiome. Nat Rev Genet 13: 47–58.
33. Ley RE, Turnbaugh PJ, Klein S, Gordon JI (2006) Microbial ecology: human gut microbes associated with obesity. Nature 444: 1022–1023.
34. Atarashi K, Tanoue T, Oshima K, Suda W, Nagano Y, et al. (2013) Treg induction by a rationally selected mixture of Clostridia strains from the human microbiota. Nature 500: 232–236.
35. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, et al. (2013) Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell Host Microbe 14: 207–215.
36. Lozupone CA, Stombaugh JI, Gordon JI, Jansson JK, Knight R (2012) Diversity, stability and resilience of the human gut microbiota. Nature 489: 220–230.