Title: Next-generation sequencing as a clinical laboratory tool for describing different microbiotas: an urgent need for future paediatric practice

Short Title: Next-generation sequencing different microbiotas in paediatrics

Authors:

Richard Hansen[1], Mona Bajaj-Elliott[2], Georgina L. Hold[3], Konstantinos Gerasimidis[4], Tariq H. Iqbal[5], Gregory Amos[6], Linda V. Thomas[7], Julian R. Marchesi[8].

On behalf of Gut Microbiota for Health expert panel of British Society of Gastroenterology.

Affiliations:

1. Royal Hospital for Children, Paediatric Gastroenterology, Glasgow, United Kingdom
2. Department of Infection, Immunity and Inflammation, Institute of Child Health, University College London, London, United Kingdom
3. Microbiome Research Centre, St George and Sutherland Clinical School, University of New South Wales, Sydney, Australia
4. Human Nutrition, School of Medicine, College of Medical, Veterinary and Life Sciences, University of Glasgow, Glasgow, United Kingdom
5. Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, United Kingdom

6. National Institute for Biological Standards and Control, South Mimms, United Kingdom
7. Gut Microbiota for Health expert panel, British Society of Gastroenterology, United Kingdom
8. Department of Metabolism, Digestion and Reproduction, Imperial College London, London, United Kingdom

Licence Statement:

Corresponding Author:

Richard Hansen, Royal Hospital for Children, 1345 Govan Road, Glasgow; richard.hansen@glasgow.ac.uk; Tel: +44 141 451 6543

Word Count: 498

Acknowledgements:

RH is supported by a Career Researcher Fellowship from NHS Research Scotland. JRM is supported by the NIHR Imperial Biomedical Research Centre.

Competing Interests:

RH has received consultancy fees and conference travel support from 4D Pharma and Nutricia. GLH has received consultancy fees and conference travel support from Ferring and Proctor & Gamble. GLH is also a member of Enterobiotix scientific advisory panel. KG has received research grants, speaker's and consultancy fees from Nestle Health Science, Nutricia-Danone, Mylan, Abbott and Baxter. THI has received speaker's fees from Vifor, Pharmacosmosos, Shield, Takeda, Roche and Ferring. JRM has received consultancy fees from Enterobiotix.

Authors Contributions:

All authors contributed equally.

Key Words:

Microbiology

Paediatrics

Microbiome

Letter Text:

We read with interest the editorial by Baralle and Ismail exploring the utility of next-generation sequencing in paediatric clinical genetics[1]. The other major use of this technology, which has emerged over the last decade, is in cataloguing complex microbial communities, for instance the different human microbiomes. The microbiome describes the diverse range of microorganisms found in and on the human body and their complex interaction network. It is generally considered as comprising viruses, bacteria, archaea and fungi. The human gut contains the most complex microbial ecosystem and has been most extensively investigated to date, particularly the gut bacterial microbiota.

Briefly, two main approaches are used to identify and describe bacterial communities using next-generation sequencing: amplicon sequencing (metataxonomics) and metagenomics[2]. Amplicon sequencing relies on PCR amplification of a single

bacterial “housekeeping” gene (the 16S rRNA gene) which is considered ubiquitous but helpfully contains both highly conserved and variable regions across species. Comparing the DNA sequence read of a section of the gene against a reference database, particularly the variable regions, allows identification of the corresponding bacterium to species level if sufficient sequence data is analysed. Next-generation sequencing allows the reading of many thousands of amplicons in parallel, allowing for its use in complex microbial communities. In contrast, metagenomic sequencing reads all the DNA present in a sample after it has been randomly broken down into fragments, hence its other name- “shotgun sequencing”. Amplicon sequencing has the benefit of focussing on bacterial information but relies on inferring genomic content data from elsewhere. Metagenomics is more comprehensive but difficult to implement in mixed source DNA samples, particularly gut mucosal biopsies where human DNA far outweighs the microbial component, because all DNA sources are sequenced together by this approach. Metagenomics also suffers from annotation problems, with a large proportion of sequence reads remaining “unknown” after analysis.

Novel studies of the gut microbiome in the paediatric population have described new microbial diagnostic and prognostic markers, and have opened novel therapeutic avenues. For example, a reduction in 11 different bacteria in faeces from children with coeliac disease are specific to that disease state[3]; a distinct faecal bacterial cluster described in children post-allogenic haematopoietic stem cell transplantation confers distinct prognostic outcomes in terms of subsequent viraemia episodes (unpublished data from author MBE); and exploration of microbiota changes during exclusive enteral nutrition treatment for Crohn’s disease[4] has led directly to the development of new solid food approaches to treating the condition[5].

If we are to embrace the diagnostic, prognostic and therapeutic revolution offered to us by a greater understanding of the microbiome, there is an urgent need to integrate microbiome analysis into clinical practice. To our knowledge, no UK centre currently offers this in an accredited clinical laboratory. Whilst there are significant financial, logistical, methodological, analytical and scientific challenges to undertaking clinical microbiota testing, it is surely time for the scientific microbiome community to move these techniques from the academic laboratory into the clinical setting, much as our colleagues in clinical genetics have already successfully achieved.

References:

1. Baralle D., Ismail V. “Next Generation Sequencing” as a diagnostic tool in paediatrics. *Arch Dis Child* 2021;**106**(1):1–2. Doi: 10.1136/archdischild-2020-320251.

2. Clegg F., Berry SH., Hansen R., Hold GL. Molecular analysis of the microbiome in colorectal cancer. In: Beaulieu J-F, editor. *Colorectal Cancer: Methods and Protocols, Methods in Molecular Biology*. Springer; 2018. p. 139–53.

3. Zafeiropoulou K., Nichols B., Mackinder M., Biskou O., Rizou E., Karanikolou A., et al. Alterations in Intestinal Microbiota of Children With Celiac Disease at the Time of Diagnosis and on a Gluten-free Diet. *Gastroenterology* 2020;**159**(6):2039-2051.e20. Doi: 10.1053/j.gastro.2020.08.007.

4. Quince C., Ijaz UZ., Loman N., Eren AM., Saulnier D., Russell J., et al. Extensive modulation of the fecal metagenome in children with Crohn’s disease

during exclusive enteral nutrition. *Am J Gastroenterol* 2015;**110**(12):1718–29. Doi: 10.1038/ajg.2015.357.

5. Svolos V., Hansen R., Nichols B., Quince C., Ijaz UZUZ., Papadopoulou RTRT., et al. Treatment of active Crohn's disease with an ordinary food-based diet that replicates exclusive enteral nutrition. *Gastroenterology* 2019;**156**(5):1354–67. Doi: 10.1053/j.gastro.2018.12.002.