Pediatric RESEARCH

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COMMENT

Hepatic Steatosis—a complex interaction of germs, genes and grub.

Vandana Jain[1], Charlotte Burford[2], Emma C. Alexander[2] and Anil Dhawan[1]

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Non-alcoholic fatty liver disease (NAFLD) has a prevalence of 7–10% among children in the developed world, making it the most common cause of chronic liver disease in this population. Its prevalence is rising concomitant with the rise in obesity.[1] NAFLD incorporates a spectrum of liver abnormalities, ranging from simple steatosis, to, non-alcoholic steatohepatitis (NASH), involving various degrees of inflammation, ballooning and fibrosis. NASH may evolve into cirrhosis, accounting for its place as the second most common listing indication for adult liver transplantation (LT) in the United States.[1] It is vital to intervene in the paediatric population to avert this outcome.

Dietary and lifestyle management in NAFLD remains commonplace, but poor compliance hinders this otherwise effective strategy. Pharmacological therapies, such as insulin sensitizers, antioxidants and hepato-protective agents, have shown mixed results and varying side effect profiles.[2] There remains a desire to identify other interventional strategies. Given increasing focus on the gut-liver axis, the gut microbiome (GM) is emerging as the new kid on the block, with revolutionary potential for hepatological conditions, including NAFLD.

We therefore, welcome Stanislawski et al.'s[3] large cross-sectional study on the role of GM in paediatric fatty liver. The authors selected magnetic resonance imaging to evaluate steatosis in a paediatric population cohort ($n = 107$), in which one-quarter were born to gestational diabetic mothers. GM was evaluated in this cohort using stool samples. The study highlights the technical challenges in the recruitment of asymptomatic children; with less than half of all children approached being able to provide a suitable stool specimen. The authors demonstrated a negative association of GM biodiversity with hepatic fat fraction (HFF). Qualitatively, genera, including *Bilophila, Paraprevotella, Oscillospira* and *Bacteroides*, are correlated with HFF.

The authors defined NAFLD, based on HFF ≥ 5%, in 8 children (7.8%). However, as highlighted in our recent review,[4] caution must be exercised when labelling children with 'NAFLD', based on hepatic steatosis only, given that steatosis can be a sign of other diseases. Also, as steatosis per se, is not an accurate prognostic factor for disease outcome, clinical applicability of the study remains uncertain. Lipopolysaccharide (LPS), the main component of gram-negative bacteria, through activation of toll-like receptor 4 (TLR-4) propagates an inflammatory signalling cascade, which can contribute to insulin resistance and TNF-α mediated fibro-inflammatory processes within the liver. The intricate interplay between the microbiome and the immune system, will likely play a role in NAFLD, with increased LPS concentrations being reported in NASH children.[5] Future researchers should consider routinely evaluating the microbiome in steatosis, in combination with inflammatory and fibrotic biomarkers, to understand the microbiome in the context of disease pathogenesis.

Increased intestinal permeability (IP), and small intestinal bacterial overgrowth (SIBO), have been consistently described in paediatric obesity, with increasing evidence to suggest their potential involvement in fatty liver phenotypes. Although altered GM composition, known as *dysbiosis*, is described, inconsistent results across studies exist. Initially, a decreased abundance of *Bacteroidetes* and increased *Firmicutes*, in obesity, was found at a phylum level. However, subsequent studies have shown different, and often, opposite results. Similarly, in paediatrics, contradictory results at phylum, genus and species level exist, not only between disease and healthy states, but also within disease states. In addition to differences in study populations (e.g. obesity, NAFLD, NASH, and in this study, hepatic steatosis), the inherent complexities and limitations in conducting any GM study will largely contribute to inter-study variation.

Antimicrobials, medications, diet, age, genetics and ethnicity can all influence GM. An ideal microbiome study would account for all of these,[6] which is, understandably, challenging. We commend Stanislawski et al., for their efforts to account for diet. Dietary analysis, is particular relevant in GM-NAFLD studies, due to the bidirectional influence of diet on both GM, and NAFLD. As an example, a high fructose diet, is associated with hepatic steatosis, and, has also been correlated with SIBO and increased LPS.[7] Stanislawski et al. used food questionnaire tools to demonstrate a weak association between mono-saturated fat, carbohydrates and HFF, but the effect on GM was difficult to establish.[3] Dietary assessment, although technically challenging, should be attempted in any GM-NAFLD study, in order to elucidate the complex relationship between GM, diet and NAFLD. Antimicrobials can have a significant effect on the microbiome; the Human Microbiome Project excludes cases exposed to antimicrobials within six months of stool sampling, whilst others suggest the microbiome never reaches its pre-antimicrobial state.[6] In addition, commonly used medications, such as, histamine receptor antagonists and proton pump inhibitors, and of particular interest in NAFLD, metformin, can also alter the microbiome. Hence, relevant medications should be incorporated into the study design of microbiome projects. In addition to differences in study populations (e.g. obesity, NAFLD, NASH and in this study, hepatic steatosis), the inherent complexities and limitations in conducting any GM study, such

[1]Paediatric Liver, GI and Nutrition Centre and Mowatlabs, King's College Hospital, London, United Kingdom and [2]Faculty of Life Sciences and Medicine, King's College London, London, United Kingdom
Correspondence: Vandana Jain (vjain@nhs.net)

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as stool collection, storage and extraction methodology, will largely contribute to inter-study variation.

Genetic polymorphisms in the pathogenesis of paediatric NAFLD are becoming increasingly described.[8] In particular, single nucleotide polymorphisms (SNPs) in PNPLA3 and TM6SF2 genes, encoding for proteins involved in lipid metabolism, have shown increased susceptibility to paediatric NAFLD, and, association with disease severity parameters. A bidirectional GM-genetic interplay is being described, whereby, the microbiome is genetically determined, as well as acting as an environmental factor interacting with host genetics to shape phenotype. Twin studies have effectively demonstrated highly correlated microbiomes in monozygotic twins, with specific taxa being more heritable than others. Genome wide association studies (GWAS) have identified 83 associations between human loci and the microbiome.[9] Genetic mutations associated with disease, can also influence the microbiome. Mutations in the *NOD2* gene increase susceptibility to inflammatory bowel disease (IBD), and is known to participate in immune mechanisms.[9] *Enterobacteriaceae* is significantly enriched in IBD patients with *NOD2* variants, potentially suggesting proliferation of this taxa in the absence of an appropriate NOD2-initiated immune response. Further research on host genetics and microbiome interactions in NAFLD, could help optimise GM manipulation for a given host genome, to reduce disease risk.

Despite the aforementioned complexities in interpreting GM-NAFLD results, GM-directed therapeutic trials in paediatrics have produced significant improvements in ALT, BMI, glucagon-like peptide 1 (GLP-1), as well as in appearance of steatosis on ultrasound.[2] However, logically, the most efficacious therapies will be those that are targeted towards NAFLD/NASH-specific bacteria. We ideally need to identify deficiencies in diseased populations, and, engage in targeted supplementation. As with any NAFLD study, separating concurrent lifestyle changes with outcome, is an ongoing challenge.

Stanislawski et al., demonstrated that both a high and low abundance of *bacteroides*, was positively associated with HFF.[3] This suitably emphasises another dilemma within GM literature. Which taxa are good and which are bad? The truth is, we don't know, and actually most bacteria are likely to have varying spectrum of effects, depending on multiple host factors. Hence, studies are now focussing on targeting the downstream functions of GM effects, rather than the microbiota per se.[10] The 'metabolome' refers to the complete set of metabolites within a biological sample, which can communicate with host metabolism and have downstream functional effects.

Production of bile acids (BAs) and short chain fatty acids (SCFAs), are well known GM metabolomic pathways, and have both been studied in obesity and NAFLD. In addition, evidence suggests that endogenous bacterial production of alcohol may play a role in NAFLD by increasing IP.[11] Stanislawski et al. alluded to a role for BA metabolism in their study, by suggesting that *Bilophila*, which was positively associated with HFF, thrives in bile.[3] The relationship between BAs and GM is a complex and intricate.[12] GM (notably, *Clostridiales* order) is responsible for 7 alpha-dehydroxylation of primary BAs in the colon, to form secondary BAs. In turn, BAs have potent antimicrobial effects. In NAFLD, the complex role of the BA nuclear receptor, Farnesoid X receptor (FXR) in glucose and lipid metabolism, has unravelled a novel therapeutic target. FXR agonism in obese mice reduces insulin resistance and improves lipid profiles, and, a recent human trial, showed histological improvement. SCFAs are the major fermentation products of GM, mainly comprising acetate, butyrate and propionate, and have been shown to be increased in obese mice and humans. Proposed mechanisms for SCFA and obesity include, increased energy extraction from diet, and, increased intestinal absorption via glucose like peptide 2 (GLP-2).[5,12] However, on the other hand, SCFAs can reduce IP via a G-protein coupled receptor, inferring a protective role for SCFAs. Future GM-NAFLD studies should attempt to incorporate BA and SCFA measurements, to further understand the function of the microbiome.

We must strive to build on cross-sectional studies, such as Stanislawski et al., to develop large prospective longitudinal microbiome studies, in hepatic steatosis, in conjunction with genetic, dietary, metabolomic and fibro-inflammatory pathways. Specifically tailored, GM-directed therapeutics, would be our goal.

AUTHOR CONTRIBUTIONS

A.D. came up with the original idea for the manuscript. V.J. led on the first draft. A.D., C.B. and E.A. substantially contributed to all revisions, adjustment to the design and inclusion of all relevant content, and all authors gave final approval of the version to be published.

ADDITIONAL INFORMATION

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REFERENCES

1. Fleet, S. E., Lefkowitch, J. H. & Lavine, J. E. Current concepts in pediatric non-alcoholic fatty liver disease. *Gastroenterol. Clin. North Am.* **46**, 217–231 (2017).
2. Liyanagedera, S., Williams, R. P., Veraldi, S., Nobili, V. & Mann, J. P. The pharmacological management of NAFLD in children and adolescents. *Expert Rev. Clin. Pharmacol.* **10**, 1225–1237 (2017).
3. Stanislawski M. A., et al. Gut microbiota in adolescents and the association with fatty liver: The EPOCH study. Pediatr Res 2018 https://doi.org/10.1038/pr.2018.32.
4. Hegarty, R., ., & Deheragoda, M., & Fitzpatrick, E., & Dhawan, A. Pediatric Fatty Liver Disease (PeFLD): all is not NAFLD—pathophysiological insights and approach to management. *J Hepatol* **68**, 1286–1299 (2018).
5. Zhu, L., Baker, R. D. & Baker, S. S. Gut microbiome and nonalcoholic fatty liver diseases. *Pediatr. Res.* **77**, 245–251 (2015).
6. Goodrich, J. K. et al. Conducting a microbiome study. *Cell* **158**, 250–262 (2014).
7. Machado, M. V. & Cortez-Pinto, H. Gut microbiota and nonalcoholic fatty liver disease. *Ann. Hepatol.* **11**, 440–449 (2012).
8. Goyal, N. P. & Schwimmer, J. B. The genetics of pediatric nonalcoholic fatty liver disease. *Clin. Liver. Dis.* **22**, 59–71 (2018).
9. Hall, A. B., Tolonen, A. C. & Xavier, R. J. Human genetic variation and the gut microbiome in disease. *Nat. Rev. Genet.* **18**, 690 (2017).
10. Troisi, J. et al. Urinary metabolomics in pediatric obesity and NAFLD identifies metabolic pathways/metabolites related to dietary habits and gut-liver axis perturbations. *Nutrients* **9**, 1–15 (2017).
11. Guercio Nuzio, S. et al. Multiple gut-liver axis abnormalities in children with obesity with and without hepatic involvement. *Pediatr. Obes.* **12**, 446–452, http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=med8&NEWS=N&AN=27350543 (2017). Available from.
12. Leung, C., Rivera, L., Furness, J. B. & Angus, P. W. The role of the gut microbiota in NAFLD. *Nat. Rev. Gastroenterol. Hepatol.* **13**, 412–425 (2016).