Confounding effects of metformin on the human gut microbiome in Type 2 diabetes

Murphy, A.J., Yancopoulos, G.D., and Gromada, J. (2014). Cell *159*, 691–696.

Kahn, S.E., Hull, R.L., and Utzschneider, K.M. (2006). Nature *444*, 840–846.

Perl, S., Kushner, J.A., Buchholz, B.A., Meeker, A.K., Stein, G.M., Hsieh, M., Kirby, M., Pechhold, S., Liu, E.H., Harlan, D.M., and Tisdale, J.F. (2010). J. Clin. Endocrinol. Metab. *95*, E234–E239.

Rabouille, C., Malhotra, V., and Nickel, W. (2012). J. Cell Sci. *125*, 5251–5255.

Rahier, J., Guiot, Y., Goebbels, R.M., Sempoux, C., and Henquin, J.C. (2008). Diabetes Obes. Metab. *10* (*Suppl 4*), 32–42.

Rosengren, A.H., Braun, M., Mahdi, T., Andersson, S.A., Travers, M.E., Shigeto, M., Zhang, E., Almgren, P., Ladenvall, C., Axelsson, A.S., et al. (2012). Diabetes *61*, 1726–1733.

Sachdeva, M.M., and Stoffers, D.A. (2009). Mol. Endocrinol. *23*, 747–758.

Stefanovski, D., Richey, J.M., Woolcott, O., Lottati, M., Zheng, D., Harrison, L.N., Ionut, V., Kim, S.P., Hsu, I., and Bergman, R.N. (2011). PLoS ONE *6*, e18134.

Talukdar, S., Oh, Y., Bandyopadhyay, G., Li, D., Xu, J., McNelis, J., Lu, M., Li, P., Yan, Q., Zhu, Y., et al. (2012). Nat. Med. *18*, 1407–1412.

Weir, G.C., Laybutt, D.R., Kaneto, H., Bonner-Weir, S., and Sharma, A. (2001). Diabetes *50* (*Suppl 1*), S154–S159.

Confounding Effects of Metformin on the Human Gut Microbiome in Type 2 Diabetes

Adil Mardinoglu,[1,2,*] Jan Boren,[3] and Ulf Smith[3,4]
[1]Science for Life Laboratory, KTH - Royal Institute of Technology, 171 21 Stockholm, Sweden
[2]Department of Biology and Biological Engineering, Chalmers University of Technology, 412 96 Gothenburg, Sweden
[3]Department of Molecular and Clinical Medicine, University of Gothenburg, Gothenburg, Sweden
[4]Lundberg Laboratory for Diabetes Research, Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden
*Correspondence: adilm@scilifelab.se
http://dx.doi.org/10.1016/j.cmet.2015.12.012

Type 2 diabetes (T2D) is associated with dysbiosis of the gut microbiota, though diabetes treatment regimens, including metformin, may confound the results. Forslund et al. (2015) identify distinct disease and drug signatures and highlight the importance of adjusting for treatment when investigating how T2D influences the human gut microbiome.

Type 2 diabetes (T2D) is a chronic disorder characterized by elevated blood glucose levels due to a combination of insulin resistance and insufficient insulin secretion, and its prevalence is increasing at an alarming rate worldwide. This complex disease results from interactions between genetic and environmental factors, and several risk factors including age, family history, dietary habits, sedentary lifestyle, and obesity have been identified. Recent studies analyzing the gut metagenome have also indicated that dysbiosis of the human gut microbiota is associated with T2D (Karlsson et al., 2013; Qin et al., 2012). Considering that the human body contains ten times more bacteria than human cells, it is essential to determine the specific contribution of the human gut microbiome to T2D pathogenesis to allow for the development of effective treatment strategies. However, most of the earlier gut metagenomic studies linking gut microbiota composition and chronic disease have not accounted for the effects of treatment regimens on the composition of the gut microbiome. An elegant study by Forslund et al. (2015) published in *Nature* now tackles this issue and identifies specific disease and drug signatures in the human gut microbiome of T2D patients (Figure 1A).

Forslund et al. (2015) performed a meta-analysis of metagenomic data from 199 T2D patients, from whom information on antidiabetic treatment was available, and 554 non-diabetic controls, comprising Swedish, Danish, and Chinese individuals. By analyzing the dataset without stratifying for treatment regimens, they replicated the majority of previously reported results and showed a large divergence between the study populations. They then tested for the effect of diet and treatment regimens and found a significant effect only for metformin, currently one of the most widely used antidiabetic drugs. After stratifying the T2D patients according to whether or not they were treated with metformin, Forslund et al. (2015) found differences in gut taxonomic composition between metformin-untreated T2D patients and non-diabetic controls. Using a supervised learning method for classification, regression, and outlier detection, the authors were able to identify which T2D patients were treated with metformin based on their gut microbial composition. As reported in previous studies (Karlsson et al., 2013; Qin et al., 2012), the abundance of bacterial genera containing known butyrate producers was lower in metformin-untreated T2D patients than in non-diabetic controls. Conversely, the increase in Lactobacillus seen previously in T2D patients without accounting for treatment regimen was eliminated when controlling for metformin treatment.

Biological pathway analysis based on microbial gene functions showed that metformin-untreated T2D patients were enriched in genes involved in responses to oxidative stress, such as catalase, as well as ribose, glycine, and tryptophan

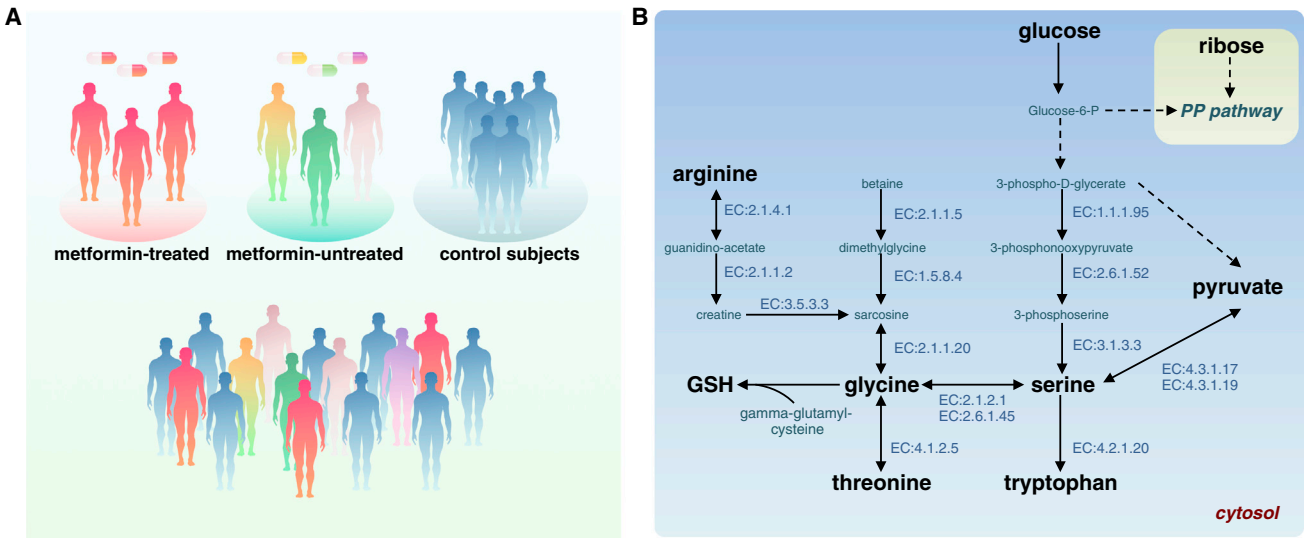

Figure 1. Stratification of the T2D Patients Based on Their Treatment Regimen

(A) Forslund et al. (2015) demonstrate that one of the most widely used antidiabetic drugs, metformin, confounds the results of the metagenomics data analysis. The authors provide evidence for the microbial-mediated effect of metformin treatment by disentangling the effects of T2D from those of metformin.

(B) Microbial genes responsible for the degradation of glycine, tryptophan, arginine, and threonine differ between metformin-untreated T2D patients and non-diabetic control subjects. The gut microbiota alters the metabolism of amino acids along the gastrointestinal tract, and thus the gut microbiota may directly affect T2D through its effect on amino acids.

degradation and depleted in genes regulating threonine and arginine degradation and pyruvate synthase capacity. Furthermore, alterations in these microbial gene modules correlated with abundance of altered bacterial taxa in these patients. To separate dysglycemia effects from T2D-specific microbial features, Forslund et al. (2015) also compared metagenomic data from 31 T1D patients with data from non-diabetic controls and showed that the majority of gut microbiota changes in metformin-untreated T2D are directly or indirectly associated with the onset or progression of T2D rather than merely an effect of dysglycemia.

To investigate whether metformin mediates its therapeutic effect partly through modulation of the microbiota, the authors compared metagenomics data from metformin-treated T2D patients to T2D patients treated with other antidiabetic medications (e.g., sulfonylurea, dipeptidyl peptidase-4 [DPP4] inhibitors, or glucagon-like peptide-1 [GLP1]). The differences were small, with multivariate analysis showing significant differences only at the bacterial family level between metformin-treated and -untreated T2D patients. However, univariate tests for specific taxa showed stronger signals than seen between metformin-untreated T2D patients and controls. Metformin-treated T2D patients from all three populations exhibited a decrease in the abundance of *Intestinibacter* spp., whereas a significant increase in the abundance of *Escherichia* spp. was found in metformin-treated T2D patients only from the Danish and Swedish populations. Functional analyses showed that gut microbiota of metformin-treated T2D patients had increased potential for butyrate and propionate production, which may in turn contribute to the beneficial effect of metformin.

Identification of taxonomic and functional microbiome signatures of metformin-untreated T2D patients will improve our understanding of the key roles played by the gut microbiome in the occurrence of T2D. Indeed, butyrate producing bacteria, which were lower in T2D patients after controlling for metformin treatment, have been shown to have health benefits (Wong et al., 2006). Moreover, Forslund et al. observed imbalances in microbial genes involved in the degradation of amino acids including glycine, tryptophan, arginine and threonine in the metformin-untreated T2D patients (Figure 1B). Consistent with these results, a recent study using genome-scale metabolic modeling to identify the metabolic differences between germ free and conventionally raised mice suggested that the gut microbiota affects the bioavailability of free amino acids to the host (Mardinoglu et al., 2015). Imbalances in the utilization of amino acids – particularly glycine, which is required for the synthesis of glutathione – may affect the biological function of the host. An earlier study using mass spectrometry and non-targeted biochemical profiling showed that of all the amino acids, plasma levels of glycine had the highest association with increased insulin sensitivity (Gall et al., 2010). Moreover, supplementation with glycine has been suggested to overcome deficiency of glutathione synthesis and improve insulin sensitivity in T2D patients (Sekhar et al., 2011). Considering that glutathione is the main detoxifying agent in the body with a vital role in regulation of the immune system, nutrient metabolism, and other important cellular events, the gut microbiota may therefore have direct involvement in the occurrence of T2D through its effect on amino acids.

The work of Forslund et al. (2015) represents a useful contribution toward our understanding of the role of the microbiome in T2D and the response to metformin. It suggests that future antidiabetic treatment strategies could target bacterial strains that cause imbalances in amino acid (e.g., glycine) metabolism. These results also provide a framework for more

detailed analysis of the gut microbiome and metabolite trafficking using genome-scale metabolic modeling, one of the common denominators in molecular systems biology (Shoaie et al., 2015). If the gut microbiota is shown to promote the onset of T2D, it may be possible to use the gut microbiota composition to determine disease progression in individual patients. This may open up the possibility for a precision medicine approach that combines information about the patient's gut microbiota with other T2D risk factors, including age, family history, and sedentary lifestyle.

REFERENCES

Forslund, K., Hildebrand, F., Nielsen, T., Falony, G., Le Chatelier, E., Sunagawa, S., Prifti, E., Vieira-Silva, S., Gudmundsdottir, V., Krogh Pedersen, H., et al. (2015). Nature *528*, 262–266.

Gall, W.E., Beebe, K., Lawton, K.A., Adam, K.P., Mitchell, M.W., Nakhle, P.J., Ryals, J.A., Milburn, M.V., Nannipieri, M., Camastra, S., et al.; RISC Study Group (2010). PLoS ONE *5*, e10883.

Karlsson, F.H., Tremaroli, V., Nookaew, I., Bergström, G., Behre, C.J., Fagerberg, B., Nielsen, J., and Bäckhed, F. (2013). Nature *498*, 99–103.

Mardinoglu, A., Shoaie, S., Bergentall, M., Ghaffari, P., Zhang, C., Larsson, E., Bäckhed, F., and Nielsen, J. (2015). Mol. Syst. Biol. *11*, 834.

Qin, J., Li, Y., Cai, Z., Li, S., Zhu, J., Zhang, F., Liang, S., Zhang, W., Guan, Y., Shen, D., et al. (2012). Nature *490*, 55–60.

Sekhar, R.V., McKay, S.V., Patel, S.G., Guthikonda, A.P., Reddy, V.T., Balasubramanyam, A., and Jahoor, F. (2011). Diabetes Care *34*, 162–167.

Shoaie, S., Ghaffari, P., Kovatcheva-Datchary, P., Mardinoglu, A., Sen, P., Pujos-Guillot, E., de Wouters, T., Juste, C., Rizkalla, S., Chilloux, J., et al.; MICRO-Obes Consortium (2015). Cell Metab. *22*, 320–331.

Wong, J.M., de Souza, R., Kendall, C.W., Emam, A., and Jenkins, D.J. (2006). J. Clin. Gastroenterol. *40*, 235–243.