An integrative cross-omics study of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis

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Project outline

Background: Non-alcoholic fatty liver disease (NAFLD) is characterized by accumulation of triglycerides (TG) in hepatic cells[1]. NAFLD may develop into non-alcoholic steatohepatitis (NASH)[2]. NAFLD and NASH are tightly associated with insulin resistance and cardiometabolic disease[3]. Recent Mendelian Randomisation (MR) studies show that the relationship between NAFLD and cardiovascular disease depends largely on cholesterol and TG levels[4,5]. A MR study of NAFLD risk alleles showed divergent metabolic effects using nuclear magnetic resonance (NMR)[6]. We recently found that hepatic steatosis and other liver-function parameters are associated with a metabolic shift in blood VLDL-and large HDL-particles, triglycerides, isoleucine and glycoprotein. This shift overlaps for 95% with that induced by a change of gut microbial diversity[7,8]. In line with our findings, exposure to microbial products were found to trigger steatosis and changes in branched-chain amino acids metabolism[9].

Hypothesis: Metabolomic changes in blood may be associated with the risk and pathological consequences of NAFLD and NASH. Integrating genetic, metagenomic and metabolomic data may elucidate (causal) pathways that may be targets for interventions. Metabolites that change as a consequence of NAFLD may be relevant as biomarkers for NASH and cardiometabolic morbidity.

Aims:

(1) Delineate the changes in metabolomics pathways that are involved in the causal pathway of NAFLD from those that are a consequence of NAFLD;
(2) Determine the role of these pathways in the risk of NASH, cardiometabolic disorders and mortality;
(3) Understand the role of the gut microbiome in the relation between metabolites, NAFLD, NASH and cardiometabolic morbidity.

Description of the work to be undertaken:

First, building upon genomic studies of the metabolome and the metabolomic studies of NAFLD conducted to date[10,11], we will perform two-sample, bi-directional MR to distinguish metabolites that change either as a cause or consequence of NAFLD[12]. We will conduct sensitivity analyses to scrutinize the MR results by: (I) performing heterogeneity tests and pleiotropy effects of the genetic variants (MR-Egger); (II) identification of specific pathways involved in the associations by pathway-based GRS

of steatosis scores, (III) exclusion of pleiotropy by other metabolites using conditional analysis based on summary statistics[13]. The deliverable of the analysis will be an atlas of metabolic changes that precede and result from NAFLD (see reference **12** for an example). **Second**, we will follow a similar MR scheme to determine whether the metabolites that change as a cause of consequence of NAFLD are also relevant for NASH, cardiometabolic disorders and mortality. We will use summary statistics of NMR metabolomics studies conducted to date and other metabolomics data that we and others are generating at present in the CHARGE consortium, UK Biobank (UKB) and elsewhere. We will also integrate epigenomic, transcriptomic and proteomic data. The fellow will conduct additional analyses, if necessary. To validate the findings of the MR and determine the clinical utility of the MR, the fellow will further determine directly the relationship of circulating metabolites that change either as a cause or consequence of NAFLD to NASH, diabetes, cardiovascular pathology and mortality in the UKB. The deliverable of the analysis will be a detailed atlas of metabolic changes that are a cause of consequence of NAFLD and predict NASH, cardiometabolic pathology and mortality. **Third,** building upon our studies and that of others linking the gut microbiome, metabolome and hepatic steatosis[7,8,9], we will evaluate the relationship of the gut microbiome with specific metabolites identified in the first and second step. Again, we will use the summary statistic of published data and those generated during the project.

Contributions: Cornelia van Duijn will be responsible for the daily supervision of the fellow. Both Joanna Howson and Cornelia van Duijn will supervise the progress of the fellow in regular meetings.

Supervisor's recent relevant publications:

1. Liu J, Lahousse L, Nivard MG, …, Stricker B, **van Duijn CM.** Integration of epidemiologic, pharmacologic, genetic and gut microbiome data in a drug-metabolite atlas. **Nat Med** 2020; 26: 110-117. PMID: 31932804
2. Vojinovic D, Radjabzadeh D, Kurilshikov A, …, **van Duijn CM**. Relationship between gut microbiota and circulating metabolites in population-based cohorts. **Nat Commun** 2019; 10:5813. PMID: 31862950
3. Liu J, Carnero-Montoro E, van Dongen J, Lent S, ……..Isaacs A, Boomsma DI, Bell JT, Demirkan A, **van Duijn CM**. An integrative cross-omics analysis of DNA methylation sites of glucose and insulin homeostasis. **Nat Commun** 2019; 10:2581. PMID: 31197173
4. Deelen J, Kettunen J, Fischer K, ……..Jukema JW, **van Duijn CM**, Slagboom PE. A metabolic profile of all-cause mortality risk identified in an observational study of 44,168 individuals. **Nat Commun**. 2019;10:3346. PMID: 31431621
5. Liu J, van Klinken JB, Semiz S, …, **van Duijn CM**, Demirkan A. A Mendelian Randomization Study of Metabolite Profiles, Fasting Glucose, and Type 2 Diabetes. **Diabetes** 2017;66:2915-2926. PMID: 28847883

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2. Ahmed, M. Non-alcoholic fatty liver disease in 2015. *World J Hepatol* **7**, 1450-1459 (2015).
3. Gruben, N., Shiri-Sverdlov, R., Koonen, D.P. & Hofker, M.H. Nonalcoholic fatty liver disease: A main driver of insulin resistance or a dangerous liaison? *Biochim Biophys Acta* **1842**, 2329-2343 (2014).
4. Brouwers, M.*, et al.* Relationship Between Nonalcoholic Fatty Liver Disease Susceptibility Genes and Coronary Artery Disease. *Hepatol Commun* **3**, 587-596 (2019).
5. Brouwers, M., Simons, N., Stehouwer, C.D.A. & Isaacs, A. Non-alcoholic fatty liver disease and cardiovascular disease: assessing the evidence for causality. *Diabetologia* (2019).
6. Sliz, E.*, et al.* NAFLD risk alleles in PNPLA3, TM6SF2, GCKR and LYPLAL1 show divergent metabolic effects. *Hum Mol Genet* **27**, 2214-2223 (2018).
7. Dina Vojinovic*, et al.* Relationship between gut microbiota and circulating metabolites in population-based cohorts. *Nature communications* **In press**(2019).
8. Liu, J., Lahousse, L. & Nivard, M.G. Integration of epidemiologic, pharmacologic, genetic and gut microbiome data in a drug-metabolite atlas. *Nature Medicine* **In press**(2020).
9. Hoyles, L.*, et al.* Molecular phenomics and metagenomics of hepatic steatosis in non-diabetic obese women. *Nat Med* **24**, 1070-1080 (2018).
10. Kettunen, J.*, et al.* Genome-wide study for circulating metabolites identifies 62 loci and reveals novel systemic effects of LPA. *Nat Commun* **7**, 11122 (2016).
11. Namjou, B.*, et al.* GWAS and enrichment analyses of non-alcoholic fatty liver disease identify new trait-associated genes and pathways across eMERGE Network. *BMC Med* **17**, 135 (2019).
12. Liu, J.*, et al.* A Mendelian Randomization Study of Metabolite Profiles, Fasting Glucose, and Type 2 Diabetes. *Diabetes* **66**, 2915-2926 (2017).
13. Deng, Y. & Pan, W. Testing Genetic Pleiotropy with GWAS Summary Statistics for Marginal and Conditional Analyses. *Genetics* **207**, 1285-1299 (2017).
14. Yengo, L.*, et al.* Meta-analysis of genome-wide association studies for height and body mass index in approximately 700000 individuals of European ancestry. *Hum Mol Genet* **27**, 3641-3649 (2018).
15. Klarin, D.*, et al.* Genetics of blood lipids among ~300,000 multi-ethnic participants of the Million Veteran Program. *Nat Genet* **50**, 1514-1523 (2018).
16. Vujkovic, M.*, et al.* Discovery of 318 novel loci for type-2 diabetes and related micro- and macrovascular outcomes among 1.4 million participants in a multi-ethnic meta-analysis. *medRxiv*, 19012690 (2019).
17. Wheeler, E.*, et al.* Impact of common genetic determinants of Hemoglobin A1c on type 2 diabetes risk and diagnosis in ancestrally diverse populations: A transethnic genome-wide meta-analysis. *PLoS Med* **14**, e1002383 (2017).
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21. Sun, B.B.*, et al.* Genomic atlas of the human plasma proteome. *Nature* **558**, 73-79 (2018).
22. Bonder, M.J.*, et al.* Disease variants alter transcription factor levels and methylation of their binding sites. *Nat Genet* **49**, 131-138 (2017).
23. Watanabe, K.*, et al.* A global overview of pleiotropy and genetic architecture in complex traits. *Nat Genet* **51**, 1339-1348 (2019).
24. Wang, J.*, et al.* Meta-analysis of human genome-microbiome association studies: the MiBioGen consortium initiative. *Microbiome* **6**, 101 (2018).