Harnessing environmental redox stresses to identify new metabolic signalling pathways causing Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD)

Oxford supervisors: Prof Keith Channon[1] and Prof Leanne Hodson[2]

Novo Nordisk supervisors: Dr Giorgio Caratti[3]

Departments: 1. Division of Cardiovascular Medicine, Radcliffe Department of Medicine
2. Oxford Centre for Diabetes, Endocrinology and Metabolism
3. Novo Nordisk Research Centre Oxford

Background: The increasing prevalence of Metabolic Dysfunction-Associated Steatotic Liver Disease (MASLD) is a rapidly increasing global health burden, associated with lifestyle factors (e.g. diet), obesity and cardiometabolic disease. MASLD affects ~30% of the global population and will rise to ~50% by 2040. However, the specific mechanisms linking MASLD to diet and obesity remain unclear. Whilst BMI is a strong predictor of MASLD, specific dietary and other environmental factors, and distinct genetic mechanisms, are important factors in MASLD susceptibility. Importantly, MASLD is a key risk factor for the development of Metabolic dysfunction-Associated Steatohepatitis (MASH), which eventually leads to liver failure and potentially the development of hepatocarcinoma.

In humans, specific mechanisms have evolved to adapt humans to exposure to environmental and dietary 'stresses'. One evolutionarily ancient mechanism, conserved from bacteria to eukaryotes, is the ability to detoxify inorganic arsenic. Inorganic arsenic (iAs) is a globally important environmental redox stress, as many regions of the world have high levels of iAs in drinking water, and many staple foods, particularly those cultivated in ground-derived water, such as rice, can be high in iAs. High levels of iAs exert toxic effects on cells via redox reactions with critical signalling nodes such as the thioredoxin-glutathione systems and redox modification of -SH groups in proteins. iAs is detoxified by methylation, generating methylAs compounds that are excreted and do not accumulate in tissues. The key iAs methylating enzyme in humans is arsenic 3-methyl transferase (AS3MT), with close homology across all mammals.

Independently of its known role in detoxification of iAs, we discovered that *As3mt* is a highly differentially-expressed gene in screens of altered redox regulation (1, 2), raising the possibility that AS3MT may have a more general roles in redox signalling. Furthermore, we found that in *As3mt* knockout mice, high fat/high sugar feeding to induce obesity leads to a modest increase in body weight gain and impaired glucose intolerance, but a massive difference in the development of fatty liver (Figure, below). Work by our collaborator reveals that *As3mt* KO mice have significant differences in plasma and urinary metabolomic, lipidomic and carbohydrate metabolic profiles (e.g differences in phosphatidylcholines, cytidine, acyl-carnitine, hippuric acid, acetylglycine, urea, L-sorbose, galactonic acid, gluconic acid). Some of the differentially altered metabolites are related to the function of other methyltransferases, independent of iAs treatment, indicating that As3mt may be involved in other cellular processes, beyond iAs methylation (3). However, the relevance of these findings in mouse models to human MASLD pathogenesis remains unclear.

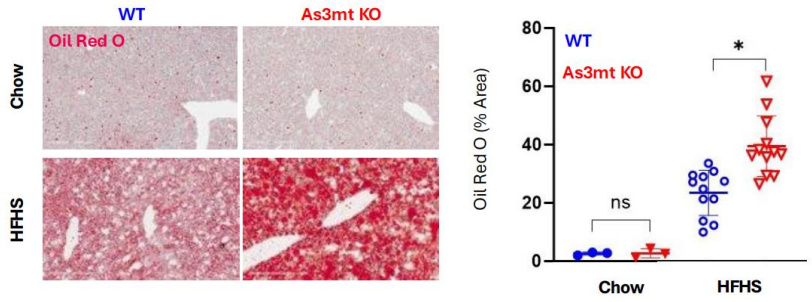

Figure: As3mt deletion exacerbates fatty liver in high fat, high sugar (HFHS) fed mice. Wild type (WT) and As3mt knockout (KO) mice were fed either chow or HFHS diet for 16 weeks. Livers were harvested and tissue sections were stained with Oil Red O to visualise lipid content (left panel), and quantified from n=12 animals in each group (right panel; * denotes p<0.05)

Thus, exposure to iAs, the ability to detoxify iAs and other related redox stresses, and the specific role of *AS3MT*, are exemplar systems to understand metabolic pathways contributing to the development of MASLD. Given the global importance of low-level environmental redox stresses in populations consuming drinking water or foodstuffs with iAs or other contaminants, understanding these pathways may open up major new opportunities for intervening in MASLD or MASH.

Common inherited genetic variation in the 10q24.32 region (containing *AS3MT*, including SNPs rs9527 and rs11191527) is associated with altered responses to iAs exposure – both in terms of biochemical markers of iAs methylation, and susceptibility to iAs effects, in studies in some populations exposed to high iAS (e.g. Bangladesh, South Amercia), but require more systematic validation in larger population groups that are relevant to the global burden of MASLD. Nevertheless, these findings highlight the potential to use genetic instruments to test the causal role of *AS3MT* in human disease, using experimental medicine studies of selected genotyped individuals, and in Mendelian randomization studies of large cohorts.

We Hypothesise that AS3MT in liver is protective in the development of MASLD, driven by non-canonical redox- and methylation-dependent processes that are currently under-recognised as causal pathways in MASLD. We propose that metabolomic and transcriptional profiling of AS3MT-deficient hepatocytes will identify new unknown drivers and/or effectors of redox causal pathways in MASLD, that can be triaged and validated by human genetic studies.

In **Aim 1**, we will test the requirement for *AS3MT* in human hepatocytes exposed to MASLD-related stimuli such as lipids/fatty acids, high glucose and inflammatory cytokines. Comparison with high iAs will be used to determine the relative importance of iAs-dependent vs. independent effects, measuring iAs levels and methylated-As species via our collaborators (4). We will use human hepatocyte culture models that are well-established in previous Novo Nordisk-Oxford projects (5), knocking down *AS3MT* using siRNA. We will determine the effects of *AS3MT* knockdown on hepatocyte transcriptomic profile, lipidomic and metabolomic responses.

In **Aim 2**, we will collaborate with the Computational Precision Health department and the Genetics Centre of Excellence (CoE) at Novo Nordisk to explore the causal relationships between AS3MT and MASLD or MASH. Since liver conditions are largely under-diagnosed, we will use derived phenotypes for liver diseases as surrogates, such as cirrhosis and liver enzymes. We will use genetic and liver transcriptomic data from GTEx, and proteomic data from STARNET (6), to identify genetic proxies for AS3MT activity and effects. These genetic instruments will then be leveraged in genome-wide association studies (GWAS) of environmental arsenic exposure in drinking water (7), as well as liver conditions and functions, including MASLD, MASH (using in-house GWAS data from Novo Nordisk), cirrhosis (6), chronic elevation of ALT (8), and other liver enzymes from UK Biobank, FinnGen, and Japan Biobank, to infer causal relationships through Mendelian randomization and genetic colocalization (statistical genetics pipelines developed in the Genetics CoE at Novo Nordisk). The

variants with a shared association between AS3MT gene expression/protein and cardiometabolic diseases/traits will be prioritised in Aim 3. We will explore other real world evidence to investigate associations between levels of Arsenic in drinking water (as mandated by the WHO) and regional diagnoses of MASLD or MASH. We will apply Data Fusion approaches to link publicly available data on local area level arsenic exposure in drinking water to geographical information in large cohort data (e.g., UK Biobank) to explore potential effects of population level arsenic on disease incidence. Analyses will explore associations between diagnostic codes of arsenic exposure (ICD 10 Z77.010) and subsequent MASLD or MASH, in large, routinely collected electronic health records (e.g, CPRD).

In **Aim 3**, we will investigate, in vivo, in humans how *AS3MT* genetic variants alter the hepatic and systemic metabolic responses during a switch in metabolic state. Using the Oxford Biobank, we will identify participants with specific *AS3MT* haplotypes, inviting those homozygous for the variant *AS3MT* alleles known to alter AS3MT expression and/or activity, compared with age, sex and BMI-matched controls. Data from previous studies indicate a variant allele frequency of ~16% (e.g. for rs9527), giving a homozygote frequency of ~2.6%, i.e. 260 individuals in the Oxford Biobank of ~10 000 participants. The effect size of the rs9527 variant allele is 1.8 sds for %iAs and 2.0 sds for %methylAs levels, enabling adequate power to detect functional consequences in groups of 15 people with variant *AS3MT* genotype vs. common allele control subjects. Moreover, in collaboration with the Genetics CoE, we will explore the effects of *AS3MT* mutations on a spectrum of human metabolic conditions and diseases using large-scale biobanks and multi-omics data.

We will measure metabolic readouts of liver metabolism and function in the fasting and postprandial state using techniques that are well-established in our groups, and in previous NNRCO-Oxford collaborative projects (6, 7, 8). Briefly, liver fat content will be assessed by magnetic resonance spectroscopy (MRS) at OCMR, and stable-isotope tracers will be used to assess hepatic de novo lipogenesis (DNL) in the fasting and postprandial states. Participants will be fed a mixed-test meal containing a ^{13}C-labelled fatty acid, and the appearance of ^{13}C will be measured in markers of esterification and FA oxidation pathways (9). Blood and breath samples will be collected regularly over a 6 h period for measurement of relevant biochemical parameters.

Supervisor's recent relevant publications

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