**Genetic mapping of insulin sensitivity in Diversity Outbred mice**

**Aims:** The aim of this study was to identify the genetic drivers of insulin resistance – a major risk factor for the development of type 2 diabetes.

**Methods:** We performed linkage and single nucleotide polymorphism (SNP) based genetic mapping of insulin sensitivity in 670 Diversity Outbred mice. Downstream analyses were performed using 16s amplicon sequencing, mass spectrometry-based multiomics, and in-depth metabolic phenotyping.

**Results:** We identified a quantitative trait loci (QTL) for insulin sensitivity with genome-wide significance within the defensin region of chromosome 8. Within this loci, the SNP rs23754102 was suggested to be insulin sensitising, and 16s amplicon sequencing of mice carrying this variant revealed an enrichment for metabolically protective microbes including *Akkermansia muciniphila.* Furthermore, cage-mates of these mice also exhibited enhanced insulin sensitivity and increased *A. muciniphila* abundance suggesting a microbiome mediated phenotype. To further test the association between defensins and insulin sensitivity we performed proteomics on the small intestines of 7 genetically distinct mouse strains. This identified α-defensin-26 as positively contributing to whole-body insulin sensitivity and linked genetic variation in this region to alpha defensin-26 protein expression. Based on our data, and to test causality, we then supplemented the diets of mice with α-defensin-26 and performed metabolic phenotyping and microbiome profiling. This revealed striking strain-specific effects of defensin supplementation on insulin sensitivity, lean mass, insulin secretion, and bile acid metabolism.

**Conclusion:** These data illustrate the importance of considering biological variation in developing therapeutics and provide the first evidence of a genetic link between microbiome composition and metabolic health.