Evidence for Involvement of *WDPCP* Gene in Alcohol Consumption, Lipid Metabolism, and Liver Cirrhosis

Felix O'Farrell [1*], MSc; Benjamin Aleyakpo[2*], MSc, PhD; Rima Mustafa [3,4*], MD, PhD; Xiyun Jiang [1*], MSc; Rui Climaco Pinto[3, 5*], PhD; Paul Elliott [3-8], MB, PhD; Ioanna Tzoulaki [3,9], PhD; Abbas Dehghan [3], MD, PhD; Samantha H. Y. Loh[10], PhD; Jeff W. Barclay[11], PhD; L. Miguel Martins[10], PhD; Raha Pazoki [1,3], MD, PhD

1. Cardiovascular and Metabolic Research Group, Division of Biosciences, Department of Life Sciences, College of Health and Life Sciences, Brunel University, London, UB8 3PH, United Kingdom.

2. The Francis Crick Institute, London, NW1 1AT, United Kingdom.

3. School of Public Health, St Mary's campus, Norfolk Place, London W2 1PG, United Kingdom.

4. UK Dementia Research Institute, Imperial College London, Exhibition Road, London, SW7 2AZ, United Kingdom.

5. MRC Centre for Environment and Health, Department of Epidemiology and Biostatistics, School of Public Health, St Mary's campus, Norfolk Place, London W2 1PG, United Kingdom.

6. British Heart Foundation Centre of Research Excellence, Imperial College London, Du Cane Road, W12 0NN, United Kingdom.

7. National Institute for Health Research, Imperial Biomedical Research Centre, Imperial College London, Exhibition Road, London, SW7 2AZ, United Kingdom.

8. Health Data Research UK at Imperial College London, Exhibition Road, London, SW7 2AZ, United Kingdom.

9. Centre for Systems Biology, Biomedical Research Foundation, Academy of Athens, Athens, Greece.

10. MRC Toxicology Unit, University of Cambridge, Gleeson Building, Tennis Court Road, Cambridge CB2 1QR, UK.

11. Department of Molecular Physiology and Cell Signalling, Institute of Systems, Molecular and Integrative Biology, University of Liverpool, Liverpool L69 3BX.

* These authors contributed equally

Corresponding author:

Raha Pazoki, Division of Biomedical Sciences, Department of Life Sciences, College of Health and Life Sciences, Brunel University, London, UB8 3PH, United Kingdom

Email: raha.pazoki@brunel.ac.uk

Abstract:

Alcohol consumption continues to cause a significant health burden globally. The advent of genome-wide association studies has unraveled many genetic loci associated with alcohol consumption. However, the biological effect of these loci and the pathways involved in alcohol consumption and its health consequences such as alcohol liver disease (ALD) remain to be elucidated. We combined human studies with model organisms *Drosophila melanogaster* and *Caenorhabditis elegans* to shed light on the molecular mechanisms underlying alcohol consumption and the health outcomes caused by alcohol intake. Using genetics and metabolite data within the Airwave study, a sample of police forces in the UK, we performed several analyses to identify changes in circulating metabolites that are triggered by alcohol consumption. We selected a set of genes annotated to genetic variants that are (1) known to be implicated in alcohol consumption, (2) are linked to liver function, and (3) are associated with expression (cis-eQTL) of their annotated genes. We used mutations and/or RNA interference (RNAi) to suppress the expression of these genes in *C. elegans* and *Drosophila*. We examined the effect of this suppression on ethanol consumption and on the sedative effects of ethanol. We also investigated the alcohol-induced changes in triacylglycerol (TGA) levels in *Drosophila* and tested differences in locomotion of *C. elegans* after acute exposure to ethanol. In human population, we found an enrichment of the alcohol-associated metabolites within the linoleic acid (LNA) and alpha linolenic acid (ALA) metabolism pathway. We further showed the effect of *ACTR1B* and *MAPT* on locomotion *in C. elegans* after exposure to ethanol. We demonstrated that three genes namely *WDPCP, TENM2* and *GPN1* modify TAG levels in *Drosophila*. Finally, we showed that gene expression of *WDPCP* in human population is linked to liver fibrosis and liver cirrhosis. Our results underline the impact of alcohol consumption on metabolism of lipids and pinpoints *WDPCP* as a gene with potential impact on fat accumulation upon exposure to ethanol suggesting a possible pathway to ALD.

Introduction:

Alcohol consumption is a major public health concern and is responsible for over 5% of the global burden of disease [1]. It has been known for a long time that excessive drinking leads to a range of pathologies including alcohol liver disease (ALD). Some of the known biological changes involved in ALD are acceleration of hepatic lipogenesis in which *ADH* and *CYP2E1* genes are known to play a role [2].

Advances in genomics within the last two decades have resulted in a boost in our understanding of the mechanism of diseases through agnostic approaches such as genome-wide association studies (GWAS) that revealed numerous genetic loci linked to complex diseases. Recently, through GWAS we have identified genetic variants in the form of single nucleotide polymorphisms (SNPs) that are associated with alcohol consumption [3] as well as with circulating liver enzymes [4] in the European populations. Some of the identified genes (e.g. *ADH*, *KLB*, *DRD2*) have been investigated for better understanding of their involvement in alcohol consumption and health consequences such as ALD. However, the biological effect of most alcohol associated genes remain to be elucidated.

In this study, we aimed to shed light into the biological pathways involved in alcohol consumption and its effects towards ALD. We investigated metabolic changes in alcohol consumption and the pathways involved. We subsequently investigated biological effect of alcohol associated genes in ethanol exposed *C. elegans* and *Drosophila* to generate knowledge that could ultimately be used to develop new strategies for the prevention and treatment of ALD.

Methods:

- Population analysis

In the current study, we followed a multi-stage approach using population-based studies and model organisms to better understand pathways involved in alcohol consumption and its health consequences. We used data from the Airwave Health Monitoring Study [5], an occupational cohort of 53,116 police officers and staff ages 18 years and over across the UK. The Airwave Health Monitoring Study was approved by the National Health Service Multi-site Research Ethics Committee (MREC/13/NW/0588). Detailed information about the Airwave population, metabolic assays, data processing, metabolite annotation as well as genotyping and imputation is included in the supplementary methods.

We performed an agnostic association analysis between alcohol consumption and targeted metabolites and then investigated causality of the alcohol-metabolite associations and performed pathway analysis on the metabolites identified to have changed in the body due to alcohol consumption. We investigated known alcohol associated genes in *C. elegans* and *Drosophila* to pinpoint the genes involved in health consequences of alcohol consumption. Finally, we performed secondary analysis on the main results using bioinformatics approaches to provide a more comprehensive picture of the biological role of the genes involved in alcohol consumption. See below for a more detailed description of the above mentioned methods.

Analysis of the Metabolome. We analysed the association between alcohol consumption and circulating metabolites within the Airwave sample. Metabolomics features were obtained from data acquired by the National Phenome Centre (NPC) using liquid-chromatography / mass spectrometry), covering a wide range of hydrophilic and lipid metabolite classes. We excluded unannotated features from the analysis. To determine over-representation of alcohol-associated metabolites in known metabolic pathways, we performed pathway enrichment analysis using all annotated alcohol-associated metabolomics features. To this end, the Kyoto Encyclopedia of Genes and Genomes (KEGG) [6] and The Small Molecule Pathway Database (SMPDB) [7] were used. In addition, we performed association analyses between our alcohol-associated metabolites and known alcohol-associated SNPs and further performed causal inference analyses on these results using the Inverse variance weighted two-sample Mendelian randomization (MR) analysis. The aim of the MR analysis was to identify changes in circulating metabolites caused by alcohol consumption. MR is a causal inference method in observational studies that mimics randomised controlled trials (RCTs) by taking advantage of random assortment of alleles at conception. It uses instrumental variables (i.e. genotype status) that are robustly associated with an exposure of interest as a randomisation tool occurring naturally at conception [8].

Gene Selection for model organisms. We selected a list of 105 alcohol-related SNPs from recently conducted GWAS of alcohol consumption [9, 10]. SNPs were selected if they presented a *P*-value lower than a GWAS significance threshold of 5×10^{-8} in their association with alcohol consumption. We sought for pleiotropic effect of these SNPs with liver function, a direct health burden of alcohol consumption, using our recently published GWAS of circulating liver enzymes [4]. To account for multiple testing, a corrected *P*-value threshold of <0.00048 was used for the association with liver enzymes. This corresponds to a nominal *P*-value (0.05) that has been adjusted for the number of alcohol-related SNPs (n=105) using the Bonferroni method [11]. Of the 105 SNPs associated with alcohol consumption, 43 SNPs were associated with at least one of the three liver enzymes alanine transaminase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (GGT). We additionally used eQTL data within the GTEx database [12] to identify SNPs demonstrating effect on gene expression of their nearest genes (a cis-eQTL effect). We eventually selected 24 SNPs that showed evidence of a statistically significant eQTL effect (**Table 1**).

Ortholog Selection. The 24 SNPs with cis-eQTL effect with their annotated genes were matched to their orthologs in Drosophila using FlyBase (DIOPT online tool version 8.5/9.0; beta; http://www.flyrnai.org/diopt). To ensure selection of the most credible orthologs, we used scores calculated in FlyBase. This database provides a number of approaches that support the gene-pair relationship out of a total number of tools that computed relationships between *Homo sapiens* and *Drosophila*. Genes with a score of >12 were shortlisted for further analysis in *Drosophila*. Nineteen *Drosophila* orthologs were identified of which eight had a score ≥ 12 including *ARPC1B* (*arpc1*), *ACTR1B (arp1), GPN1* (*CG3704*), *WDPCP* (*frz*), *MLXIPL* (*mondo*), *SLC4A8 (ndae1), SCN8A* (*para*), and *TENM2* (*Ten-m*).

To identify potential worm orthologs, the SNPs with cis-eQTL effect with their annotated genes were sought for their worm orthologs within WormBase (https://wormbase.org/).

BLASTp analysis results of human protein sequence against *C. elegans* protein database were obtained in WormBase version WS280 using data from *C. elegans* Sequencing Consortium genome project (PRJNA13758). The human protein sequence for the protein encoded by the genes under this study was extracted from UniProt (https://www.uniprot.org/). Worm genes with best alignment with the human protein sequence indicated by an Expect value (E value) $< 1 \times 10^{-5}$ and the highest BLASTp score (bits) were moved forward. The E value represents the number of alignments that could be found in similarity to the protein sequence by chance. Using WormBase, 13 worm orthologs were identified including *ACTR1B* (*arp*-1), *ARPC1B* (*arx-3*), *GPN1* (*gop-2*), *MLXIPL* (*mml-1*), *STAT6* (*sta-1*), *TENM2* (*ten-1*), *KIF26A* (*vab-8*), *SLC4A8* (*abts-1*), *MAPT* (*ptl-1*) and *SCN8A* (cca-1). We excluded *KLB* (*klo-1*), *DRD2* (*dop-2*), and *ADH1B* (*adh-5*) from the *C. elegans* experiments as similar studies investigating these genes already exist [13-15].

We did not identify any fly or worm orthologs for *NUCKS1*, *GCKR*, *BDNF*, *FTO*, *TCAP*. Additionally, no worm ortholog was found for *WDPCP*, *MSANTD1*, *MYBPC3*, *PMFBP1*, *TNFSF13*, and *ACSS1*.

- *Drosophila*

Genetics and Drosophila melanogaster strains. All *Drosophila* stocks and crosses were maintained on standard cornmeal agar media at 25°C on 12/12 hour light/dark cycles. The following strains were used as positive control: w^{berlin}; $hppy^{17\text{-}51}$; + (*hppy* mutant) and w^{berlin}; $hppy^{17\text{-}51}$; *hppy III* (*hppy* mutant with a rescue genomic construct) (gifts from Prof. Ulrike Heberlein, Janelia Research Campus, Virginia, USA), RNAi lines were obtained from Bloomington *Drosophila* Stock Center. All lines used were backcrossed to w^{1118} or $[v]^{w1118}$ (RNAi lines). All RNAi expressions were driven by *da*Gal4. All the experiments on adult flies were performed using males.

Drosophila Ethanol Consumption Assay. The CApillary FEeder (CAFE) assay [16] was used to measure Ethanol consumption. Eight male flies were placed into an experimental vial (8 cm height, 3.3 cm diameter) containing 6 microcapillary tubes (BRAND® disposable BLAUBRAND® micropipettes, intraMark, BR708707, with 1 µL marks), each containing 5 µL of liquid food. Liquid food was prepared by dissolving 50 mg of yeast granules in 1 ml of boiling water by vortexing, followed by brief centrifugation. Then, 40 mg of sucrose (Sigma–Aldrich, 84097) was added to 800 µL of the dissolved yeast mixture, followed by vortexing. The microcapillary tubes were filled with liquid food up to the 5 µL mark. Ethanol food consists of normal food supplemented with 15% Ethanol. Each experiment consisted of 5 experimental vials per genotype with each vial containing normal food (3 capillaries) and Ethanol food (3 capillaries). The flies were acclimatised in the experimental vial without any food for 2 hours prior to the start of the experiment. This step was also used to incentivise the flies to eat once the food was introduced. The experimental vials were placed in a plastic box with a cover to control humidity. The flies were allowed to feed for 19 hours, after which the amount consumed (in mm) was measured with a digital calliper (Dasqua Bluetooth Digital Calliper 12″/300 mm, 24108120). The total amount of food consumed was calculated using the formula:

Food uptake (µl) = (Σ measured distances between 3 microcapillary tubes (mm))/14.42 mm (1 µl in measured distance)/8 flies.

Drosophila Ethanol sedation Assay. Fly sedation assay was performed as previously described [17]. Briefly, 8 flies were transferred to a 25mm x 95mm transparent plastic vial in between two cotton plugs. A piece of cotton plug at the base of the vial served as a stable surface to observe the flies and another plug was used to cap the vial and deliver the ethanol. 500ul of 100% Ethanol was added to the side of the cotton plug facing the flies. Sedation was observed manually as ST50, which is the time in minutes it takes for 50% of the flies in a sample vial to become sedated. Sedation events are recorded when the flies become inactive and lay on their backs for over 10 seconds.

Drosophila TAG measurements. Eight male flies of the indicated genotypes were placed into an experimental vial as described in ethanol consumption assay, with all 6 microcapillary tubes filled with normal food (5% sucrose + 5% yeast) or ethanol food (normal food + 15% ethanol), for 2 days. TAGs were assessed through colorimetric assays using 96-well microtiter plates and an Infinite M200Pro multifunction reader (TECAN). The assays were performed as previously described [18]. Briefly, flies were homogenised in 110 µl of PBS + 0.05% Tween 20 (PBST) for 2 min on ice and immediately incubated at 70 °C for 10 min to inactivate endogenous enzymatic activity. A 35 µL fly homogenate sample and a glycerol standard (Sigma, no. G7793) were incubated together with either 35 µL of PBST (for free glycerol measurements) or 35 µL of TAG reagent (Sigma, no. T2449, for TAG measurements) at 37 °C for 60 min. After 3 min of centrifugation at full speed, 30 µL of each sample was transferred into a clear-bottom plate (two technical replicates per biological sample) together with 100 µL of free glycerol reagent (Sigma–Aldrich, F6428) and incubated at 37°C for 5 min. TAG absorbance was divided by the protein concentration of the respective sample, which was measured by Bradford assay (Sigma–Aldrich, B6916).

- *C. elegans*

Nematode strains and culture. All *C. elegans* strains were cultured on nematode growth medium (NGM) agar plates at 20°C using *Escherichia coli* OP50 as a food source. For the wildtype worms Bristol N2 strain was used. Genes for which mutations were homozygous lethal, heterozygote mutations balanced by chromosomal translocations were instead analysed.

Nematode RNA interference (RNAi). RNAi experiments were performed on the NL2099 *rrf-3 (pk1426)* strain as previous described [13, 19]. RNAi was achieved by feeding [20] using the ORFeome based RNAi library [21]. In brief, HT115 RNAi bacterial clones were initially cultured in LB media with 100 µg/ml ampicillin and subsequently spotted in three 50 µl drops on 60 mm diameter NGM plates containing 1 mM isopropyl β-1- thiogalactopyranoside (IPTG) and 25 µg/ml carbenicillin. Plates were left to dry for 4-7 days before seeding to improve RNAi efficiency. Following seeding, five L3-L4 worms were added to each RNAi plate and cultured at 20°C until the F1 generation reached adulthood. Ethanol experiments were performed and analysed as described above and compared to worms fed with an empty RNAi feeding vector.

Nematode Behavioural assays. All ethanol experiments were performed at 20°C in a temperature-controlled room as previously described [13, 19]. Behavioural assays were conducted on young adult hermaphrodites selected from sparsely populated NGM plates. Nematodes with *loss-of-function* mutations in worm orthologues of *ACTR1B* (*arp*-1), *ARPC1B* (*arx-3*), *GPN1* (*gop-2*), *MLXIPL* (*mml-1*), *STAT6* (*sta-1*), *TENM2* (*ten-1*), *KIF26A* (*vab-8*), *SLC4A8* (*abts-1*), *MAPT* (*ptl-1*) and *SCN8A* (cca-1) were acutely exposed to ethanol and the resultant effect on rate of locomotion (thrashes per minute) was quantified in Dent's solution (140 mM NaCl, 6 mM KCl, 1 mM CaCl2, 1 mM MgCl2, 5 mM HEPES, pH 7.4 with bovine serum albumin at 0.1 mg/ml) by measuring thrashes per minute (one thrash defined as one complete movement from maximum to minimum amplitude and back) following 10 minutes exposure to the drug. Ethanol was mixed with Dent's solution at a concentration of 400 mM, which has previously been shown to produce a ~70% reduction in locomotion rate in wild-type worms [19, 22].

- Bioinformatics

Secondary analysis. To gain a better insight into the biological pathways involved in the link between alcohol consumption and liver damage, we used the genetic variants within the genes highlighted by our model organism experiments and performed a series of secondary analysis using human data. We explored publicly available data from the UK Biobank deposited in the Edinburgh Gene Atlas [23] using which we sought Phewas (Phenome-wide association analysis) databases to obtain association results between the genetic variants and 778 traits. We additionally used the genes highlighted by our model organism experiments to assess the causal effect of gene expression on liver conditions. Within these genes, the SNPs that have been identified to have cis-eQTL effect within the previously published studies were selected and used as MR instrument against liver conditions within the *twosampleMR* package in R.

Statistical analyses. Within the Airwave study sample, we performed a linear regression to study the association of alcohol consumption with each of the metabolomic features (Metabolome-wide association study; MWAS). We adjusted the statistical analysis for age, sex, smoking status, and salary class. To account for multiple testing and the high degree of correlation in metabolomics datasets, we used a permutation-based method to estimate the significance level of the associations [24, 25]. For each metabolomics platform, a *P*-value threshold equivalent to adjusting to a 5% Family-Wise Error Rate (i.e., Bonferroni method) was computed. A series of hypergeometric tests implemented in the R package MetaboAnalystR [26] was used for pathway enrichment analysis where an FDR threshold of 0.05 was used as a significance threshold. To obtain an estimation for the association of known alcohol SNPs with our alcohol-associated metabolites to be used in the MR analysis, we performed linear regression analysis within the Airwave sample (see supplementary methods for details of the GWAS on metabolomics). Linear analyses between SNPs and metabolites were conducted for each metabolomic feature with adjustment for age, sex, and genetic principal components within a subsample of Airwave that included participants with both genetic and metabolite data (N=1,970). In *C. elegans,* locomotion rate was presented normalised as a percentage of mean thrashing rate of untreated worms measured each day. All worm data were expressed

as mean ± S.E. with an N=30 individual worms. Locomotion rate significance was assessed by one-way analysis of variance (ANOVA) with post-hoc Tukey test for multiple comparisons. Statistical analyses of the *Drosophila* experimental data were performed using GraphPad Prism (www.graphpad.com). *Drosophila* data were presented as the mean values, and the error bars indicate ± SD.

Results:

To understand biological effects of alcohol consumption in human population, we investigated the circulating metabolites within the Airwave study sample using an agnostic approach which revealed association of 376 metabolites with alcohol consumption and alcohol-associated genetic variants (**Figure 1**). Pathway analysis on the identified metabolites showed that the linoleic acid (LNA) and alpha linolenic acid (ALA) metabolism pathway (LNA/ALA) within the Small Molecule Pathway Database (SMPDB) was enriched with alcohol-associated features (Pfdr=5.67 × 10^{-3}). These features included Tetracosapentaenoic acid (24:5n-3; β=0.01; 95%CI=0.008,0.012; *P*-value=4.2 ×10^{-12}), Eicosapentaenoic acid (β=0.009; 95%CI=0.007,0.011; *P*-value=2.4 × 10^{-12}), Stearidonic acid (β=0.008; 95%CI=0.006,0.01; *P*-value=2.2 × 10^{-9}), Arachidonic acid (β=0.007; 95%CI=0.005,0.009; *P*-value=9.7 × 10^{-8}) and Adrenic acid (β=0.01; 95%CI=0.008,0.012; *P*-value=9.7 × 10^{-8}). To assess the causality of these associations, we used alcohol-associated genetic variants against circulating metabolites within the Airwave sample using MR analysis (**Table 2**). This revealed possible causal association of alcohol consumption on changing circulating level for several lipid metabolites (TAGs, Diradylglycerols, Glycerophosphocholines, Sphingolipids), and an alkaloid (piperine). The most statistically significant causal association was observed with a triacylglycerol TG 60:2 (β=1.24; 95% CI=0.52,1.95; *P*-value=0.002).

We used *Drosophila hppy* mutant, described to have an increased resistance to ethanol sedation [27], as positive control in all fly experiments. *Drosophila* with knock-down of *ARPC1B* (*arpc1*), *GPN1* (*CG3704*), *WDPCP* (*frz*), *MLXIPL* (*mondo*), *SCN8A* (*para*), and *TENM2* (*Ten-m*) together with *hppy* mutant were exposed to food supplemented with 15% Ethanol in a CAFE assay. A significant difference (**Figures 2A & 2B**) was observed between ethanol consumed (μL) by flies with RNAi knockdown of *TENM2* (*Ten-m*). Following exposure to ethanol vapour, the effect on Sedation Time 50% (ST50; minute) was quantified and we observed that in comparison to control (*hppy*), RNAi knockdown of fly orthologues of *WDPCP* (*frz*) showed a faster rate of sedation whilst *TENM2* (*Ten-m*), *GPN1* (*CG3704*), *ARPC1B* (*arpc1*) and *SCN8A* (*para*) showed a slower rate of sedation indicated by a higher ST50. *WDPCP* (*frz*) knockdown flies showed reduced TAG levels after exposure to ethanol food whilst *TENM2* (*Ten-m*) and *GPN1* (*CG3704*) knockdown flies showed a reduction in TAG levels with normal food (**Figure 2C**).

C. elegans loss-of-function mutants of *TENM2* (*ten-1*), *KIF26A (vab-8)*, *SLC4A8 (abts-1)* and *SCN8A (cca-1)* showed significant differences in basal locomotion rate (**Supplementary Figure S1A).** After acute exposure to ethanol in *C. elegance loss-of-function* mutants, significant differences were identified in normalised locomotion rate of *ACTR1B* (*arp*-1), *ARPC1B* (*arx-3*) and *MAPT* (*ptl-1*) in comparison with Bristol N2 wild-type worms (**Figure 3A**). RNAi knockdown of these genes confirmed that in comparison to controls, RNAi knockdown of

worm orthologues of *ACTR1B* (*arp*-1) and *MAPT* (*ptl-1*) and *ARPC1B* (*arx-3*) did not have any effect on basal locomotion rate (**Supplementary Figure S1B)** but RNAi knockdown of *ACTR1B* (*arp*-1) and *MAPT* (*ptl-1*) phenocopied the *loss-of-function* mutations after exposure to ethanol (**Figure 3B**).

Secondary analysis. The Phewas analysis (**Table 3**) on the three genetic variants within *WDPCP*, *TENM2*, and *GPN1* showed a link between rs10078588 (*TENM2*) and food and liquid intake (beef, oily fish, fresh fruit, pork, bread, alcohol, water, coffee, salt) as well as trunk fat percent and smoking. rs13032049 (*WDPCP*) was linked to fresh fruit intake, salt and coffee intake, adiposity, angina pectoris, myocardial infarction, and depression. rs2178197 (*GPN1*) was linked to hypertension, hematologic traits, and body impedance. All three SNPs showed association with disorders of lipoprotein metabolism and other lipidaemias (ICD code E78) within the UK Biobank (**Table 4**). We finally performed a Mendelian randomization analysis on expression of *WDPCP* gene and liver conditions (**Table 5**) and identified a link between expression of this gene with liver fibrosis and cirrhosis (β=-0.20; 95% CI=-0.39, -0.01; *P*-value=0.04) and liver and bile duct cancer (β=0.0003; 95% CI=3.27×10^{-05}, 5.85×10^{-04}; *P*-value=0.02). We also observed a suggestive link with the changes in liver fatty acid-binding protein, and liver enzyme levels (**Table 5**).

Discussion:

In this study we used data from humans, *C. elegans*, and *Drosophila* and identified a link between genes implicated in alcohol consumption and lipid metabolism. We identified that alcohol consumption changes the metabolites within linoleic acid (LNA) and alpha linolenic acid (ALA) metabolism pathway (LNA/ALA) and demonstrates causal effect on changes in several lipid metabolites. We highlighted that change of function of the genes implicated in alcohol consumption leads to changes in ethanol consumption, sedation after exposure to ethanol vapor, and changes in accumulation of fat in *Drosophila* as well as changes in locomotion rate after exposure to ethanol in *C. elegans.* Our results demonstrate that three alcohol implicated genes namely *WDPCP* (*frz*), *TENM2* (*Ten-m*), and *GPN1* (*CG3704*) might be involved in fat accumulation.

In our study, we used alcohol associated genetic variants to (1) explored the alcohol-induced biological changes in human metabolites and (2) explore alcohol-induced biological effect of genes annotated to alcohol associated genetic variants in *C. elegans* and *Drosophila melanogaster*. Of alcohol implicated genes that we investigated in *C. elegans*, *ACTR1B* (*arp*-1) and *MAPT* (*ptl-1*), show significant effects on the worms' locomotion upon acute exposure to ethanol. In addition, *TENM2* (*Ten-m*) shows significant effects on ethanol consumption in *Drosophila* and apart from *MLXIPL* (*mondo*), RNAi lines for all the genes investigated in *Drosophila* change the time to sedation from ethanol.

The observed effect of *WDPCP* (*frz*) knockdown on the changed TAG levels occurred under exposure to ethanol and followed similar patterns as *hppy* mutants. This implies that minimizing alcohol consumption could reduce fat accumulation and thus reduce the risk of ALD. In our further MR analysis, we used publicly available databases derived from GWAS in human population and showed a link between the gene expression of *WDPCP* and liver

fibrosis and liver cirrhosis. The analysis also showed a suggestive link with the liver fatty acid binding protein that is involved in the metabolism of lipids [28]. This evidence suggests that *WDPCP* might be an important gene involved in the pathway between alcohol consumption, accumulation of fat and liver fibrosis. This could have public health implications in terms of identification of high-risk groups and targeting preventive measures as well as drug development. More studies in vivo and in vitro are needed to focus on *WDPCP* and provide more details on its role in lipid metabolism and liver pathologies.

Changes in TAG levels of *Drosophila* occurred with exposure to normal food rather than ethanol for RNAi knockdown of *TENM2* (*Ten-m*) and *GPN1* (*CG3704*) indicating that loss of function of these genes could have a direct role in accumulation of fat in liver independent of exposure to ethanol. RNAi knockdown of both genes shows increased tolerance to sedative effect of ethanol which could justify the effect of these genes on a more frequent alcohol consumption in human, possibly due to alcohol tolerance. *GPN1* is located on chromosome 2p23.3 and the encoded protein is implicated in regulation of TGFβ superfamily signaling [29] that is demonstrated to play a role in obesity [30], accumulation of fat in the liver [31] as well as regulation of *ADH1* gene that enhances alcohol-induced liver damage and lipid metabolism [32]. The existing evidence alongside our findings on the role of *GPN1* in alcohol consumption and lipid metabolism in *Drosophila* implies that *GPN1* might play a role upstream of TGFβ in the regulation of metabolism of alcohol and lipids. Further studies are needed to highlight the relationship between *GPN1* and TGFβ in alcohol consumption and alcohol-induced liver damage.

TENM2 (*Ten-m*) is located on chromosome 5q34, and the encoded protein is involved in cell adhesion [33]. *TENM2* is found to be highly enriched in white adipocyte progenitor cells [34]. *TENM2* deficiency in human fat cells leads to expression of UCP1, the primary marker of brown adipose tissue [35]. Genetic variants in *TENM2* have shown to be linked to obesity [36]. Our secondary analyses confirmed an association between the genetic variant in *TENM2* and excess of food and liquid intake which suggests the link between *TENM2* and alcohol consumption could also be due to systematic increase in consumption of all food and beverages rather than alcohol alone. The evidence in this study alongside the existing literature highlights that the observed effect of *TENM2* (*Ten-m*) RNAi knockdown on the changes in TAG levels in *Drosophila* could potentially be related to biological pathways implicated in adipose tissue rather than pure liver-related pathways.

One strength of our study is in that we performed our analyses in human and two different model organisms that allows for a more comprehensive insight into biological mechanisms involved in the function of alcohol consumption genes under different biological scenarios. A second strength of this study is in the use of RNAi technique which provides insight into the function of genes and what biological manifestation they would have when exposed to ethanol. The third strength of our study is in the use of CAFE assay that allows for investigation of food and alcohol consumption in *Drosophila* in a more controlled environment. In our CAFE assay, each experimental box per genotype contained both normal food and ethanol food (food supplemented with 15% ethanol) providing the insects with a choice. Another strength of our study is in that in our TAG levels experiments, we made our

conclusions based on the comparisons between flies (RNAi vs. control) that were exposed to identical food and environmental conditions which increases the robustness of our conclusions. Finally, we combined the results with human studies to get better insight into the link between alcohol consumption and lipid metabolites.

Although the genetic variants used for our investigations were originally found in human studies and we also performed a metabolomics analysis between alcohol consumption and circulating metabolites in human population, the main conclusions are made based on the effect of the genes in model organisms and the results of this study might not directly generalisable to patients and the public without performing further population studies.

Conclusion:

We identified that alcohol associated genes may be involved in metabolism of lipids and accumulation of fat in liver. Our study highlights three genes *WDPCP*, *TENM2*, and *GPN1* that may be involved in accumulation of fat (in liver or adipose tissue). Of these genes *WDPCP* exhibits its effects on fat accumulation in *Drosophila* with exposure to ethanol. The gene expression of *WDPCP* in human population supports a link to liver fibrosis. Further studies are necessary to investigate the role of this gene in ALD.

Acknowledgements and sources of funding:

R.P. was supported by Rutherford Fund fellowship from the Medical Research Council (MR/R0265051/1 and MR/R0265051/2). B.A., X.J., and F.O. were supported by Rutherford Fund from Medical Research Council MR/R0265051/2. R.M. was funded by the President's PhD Scholarship from Imperial College London. PE is Director of the MRC Centre for Environment and Health and acknowledges support from the Medical Research Council (MR/S019669/1). PE also acknowledges support from the UK Dementia Research Institute, Imperial College London (UKDRI-5001), Health Data Research UK London (HDRUK-1004231) and the British Heart Foundation Imperial College London Centre for Research Excellence (BHF-RE/18/4/34215). The Airwave Health Monitoring Study was funded by the UK Home Office (780- TETRA, 2003-2018) and is currently funded by the MRC and ESRC (MR/R023484/1) with additional support from the NIHR Imperial College Biomedical Research Centre in collaboration with Imperial College NHS Healthcare Trust. R.C.P is supported by the UK Dementia Research Institute (UKDRI-5001), which receives its funding from UK DRI Ltd, funded by the UK Medical Research Council, Alzheimer's Society and Alzheimer's Research UK. Work in LMM's laboratory is supported by the UK Medical Research Council, intramural project MC_UU_00025/3 (RG94521). The views expressed are those of the authors and not necessarily those of the sponsors. We thank Prof. Ulrike Heberlein, (Janelia Research Campus, Virginia, USA) for generously providing us the hppy17-51 fly lines.

References

1. World Health O. Global status report on alcohol and health 2018: executive summary. 2018.
2. Osna NA, Donohue TM, Jr. and Kharbanda KK. Alcoholic Liver Disease: Pathogenesis and Current Management. *Alcohol research : current reviews*. 2017;38:147-161.
3. Evangelou E, Gao H, Chu C, Ntritsos G, Blakeley P, Butts AR, Pazoki R, Suzuki H, Koskeridis F, Yiorkas AM, Karaman I, Elliott J, Luo Q, Aeschbacher S, Bartz TM, Baumeister SE, Braund PS, Brown MR, Brody JA, Clarke TK, Dimou N, Faul JD, Homuth G, Jackson AU, Kentistou KA, Joshi PK, Lemaitre RN, Lind PA, Lyytikainen LP, Mangino M, Milaneschi Y, Nelson CP, Nolte IM, Perala MM, Polasek O, Porteous D, Ratliff SM, Smith JA, Stancakova A, Teumer A, Tuominen S, Theriault S, Vangipurapu J, Whitfield JB, Wood A, Yao J, Yu B, Zhao W, Arking DE, Auvinen J, Liu C, Mannikko M, Risch L, Rotter JI, Snieder H, Veijola J, Blakemore AI, Boehnke M, Campbell H, Conen D, Eriksson JG, Grabe HJ, Guo X, van der Harst P, Hartman CA, Hayward C, Heath AC, Jarvelin MR, Kahonen M, Kardia SLR, Kuhne M, Kuusisto J, Laakso M, Lahti J, Lehtimaki T, McIntosh AM, Mohlke KL, Morrison AC, Martin NG, Oldehinkel AJ, Penninx B, Psaty BM, Raitakari OT, Rudan I, Samani NJ, Scott LJ, Spector TD, Verweij N, Weir DR, Wilson JF, Levy D, Tzoulaki I, Bell JD, Matthews PM, Rothenfluh A, Desrivieres S, Schumann G and Elliott P. New alcohol-related genes suggest shared genetic mechanisms with neuropsychiatric disorders. *Nature human behaviour*. 2019.
4. Pazoki R, Vujkovic M, Elliott J, Evangelou E, Gill D, Ghanbari M, van der Most PJ, Pinto RC, Wielscher M, Farlik M, Zuber V, de Knegt RJ, Snieder H, Uitterlinden AG, Boezen HM, Franke L, van der Harst P, Navis G, Rots M, Swertz M, Wolffenbuttel BHR, Wijmenga C, Lynch JA, Jiang X, Said S, Kaplan DE, Lee KM, Serper M, Carr RM, Tsao PS, Atkinson SR, Dehghan A, Tzoulaki I, Ikram MA, Herzig K-H, Järvelin M-R, Alizadeh BZ, O'Donnell CJ, Saleheen D, Voight BF, Chang K-M, Thursz MR, Elliott P, Ballas ZK, Bhushan S, Boyko EJ, Cohen DM, Concato J, Aslan M, Zhao H, Constans JI, Dellitalia LJ, Fayad JM, Fernando RS, Florez HJ, Gaddy MA, Gappy SS, Gibson G, Godschalk M, Greco JA, Gupta S, Gutierrez S, Hammer KD, Hamner MB, Harley JB, Hung AM, Huq M, Hurley RA, Iruvanti PR, Ivins DJ, Jacono FJ, Jhala DN, Kaminsky LS, Klein JB, Liangpunsakul S, Lichy JH, Moser J, Huang GD, Muralidhar S, Mastorides SM, Mathew RO, Mattocks KM, McArdle R, Meyer PN, Meyer LJ, Moorman JP, Morgan TR, Murdoch M, Okusaga OO, Oursler K-AK, Ratcliffe NR, Rauchman MI, Robey RB, Ross GW, Servatius RJ, Sharma SC, Sherman SE, Sonel E, Sriram P, Stapley T, Striker RT, Tandon N, Villareal G, Wallbom AS, Wells JM, Whittle JC, Whooley MA, Wilson PW, Sun YV, Xu J, Yeh S-S, Connor T, Argyres DP, Hauser ER, Beckham JC, Stephens B, Aguayo SM, Ahuja SK, Pyarajan S, Cho K, Gaziano JM, Kinlay S, Nguyen X-MT, Brewer JV, Brophy MT, Do NV, Humphries DE, Selva LE, Shayan S, Whitbourne SB, Breeling JL, Romero JPC, Ramoni RB, Lifelines Cohort S and the VAMVP. Genetic analysis in European ancestry individuals identifies 517 loci associated with liver enzymes. *Nature Communications*. 2021;12:2579.
5. Elliott P, Vergnaud AC, Singh D, Neasham D, Spear J and Heard A. The Airwave Health Monitoring Study of police officers and staff in Great Britain: rationale, design and methods. *Environ Res*. 2014;134:280-5.
6. Kanehisa M and Goto S. KEGG: kyoto encyclopedia of genes and genomes. *Nucleic acids research*. 2000;28:27-30.
7. Jewison T, Su Y, Disfany FM, Liang Y, Knox C, Maciejewski A, Poelzer J, Huynh J, Zhou Y, Arndt D, Djoumbou Y, Liu Y, Deng L, Guo AC, Han B, Pon A, Wilson M, Rafatnia

S, Liu P and Wishart DS. SMPDB 2.0: Big Improvements to the Small Molecule Pathway Database. *Nucleic Acids Research*. 2014;42:D478-D484.

8. Smith GD and Ebrahim S. 'Mendelian randomization': can genetic epidemiology contribute to understanding environmental determinants of disease? *International journal of epidemiology*. 2003;32:1-22.

9. Evangelou E, Gao H, Chu C, Ntritsos G, Blakeley P, Butts AR, Pazoki R, Suzuki H, Koskeridis F, Yiorkas AM, Karaman I, Elliott J, Luo Q, Aeschbacher S, Bartz TM, Baumeister SE, Braund PS, Brown MR, Brody JA, Clarke T-K, Dimou N, Faul JD, Homuth G, Jackson AU, Kentistou KA, Joshi PK, Lemaitre RN, Lind PA, Lyytikäinen L-P, Mangino M, Milaneschi Y, Nelson CP, Nolte IM, Perälä M-M, Polasek O, Porteous D, Ratliff SM, Smith JA, Stančáková A, Teumer A, Tuominen S, Thériault S, Vangipurapu J, Whitfield JB, Wood A, Yao J, Yu B, Zhao W, Arking DE, Auvinen J, Liu C, Männikkö M, Risch L, Rotter JI, Snieder H, Veijola J, Blakemore AI, Boehnke M, Campbell H, Conen D, Eriksson JG, Grabe HJ, Guo X, van der Harst P, Hartman CA, Hayward C, Heath AC, Jarvelin M-R, Kähönen M, Kardia SLR, Kühne M, Kuusisto J, Laakso M, Lahti J, Lehtimäki T, McIntosh AM, Mohlke KL, Morrison AC, Martin NG, Oldehinkel AJ, Penninx BWJH, Psaty BM, Raitakari OT, Rudan I, Samani NJ, Scott LJ, Spector TD, Verweij N, Weir DR, Wilson JF, Levy D, Tzoulaki I, Bell JD, Matthews PM, Rothenfluh A, Desrivières S, Schumann G and Elliott P. New alcohol-related genes suggest shared genetic mechanisms with neuropsychiatric disorders. *Nature human behaviour*. 2019;3:950-961.

10. Liu M, Jiang Y, Wedow R, Li Y, Brazel DM, Chen F, Datta G, Davila-Velderrain J, McGuire D, Tian C, Zhan X, Team aR, Psychiatry HA-I, Choquet H, Docherty AR, Faul JD, Foerster JR, Fritsche LG, Gabrielsen ME, Gordon SD, Haessler J, Hottenga J-J, Huang H, Jang S-K, Jansen PR, Ling Y, Mägi R, Matoba N, McMahon G, Mulas A, Orrù V, Palviainen T, Pandit A, Reginsson GW, Skogholt AH, Smith JA, Taylor AE, Turman C, Willemsen G, Young H, Young KA, Zajac GJM, Zhao W, Zhou W, Bjornsdottir G, Boardman JD, Boehnke M, Boomsma DI, Chen C, Cucca F, Davies GE, Eaton CB, Ehringer MA, Esko T, Fiorillo E, Gillespie NA, Gudbjartsson DF, Haller T, Harris KM, Heath AC, Hewitt JK, Hickie IB, Hokanson JE, Hopfer CJ, Hunter DJ, Iacono WG, Johnson EO, Kamatani Y, Kardia SLR, Keller MC, Kellis M, Kooperberg C, Kraft P, Krauter KS, Laakso M, Lind PA, Loukola A, Lutz SM, Madden PAF, Martin NG, McGue M, McQueen MB, Medland SE, Metspalu A, Mohlke KL, Nielsen JB, Okada Y, Peters U, Polderman TJC, Posthuma D, Reiner AP, Rice JP, Rimm E, Rose RJ, Runarsdottir V, Stallings MC, Stančáková A, Stefansson H, Thai KK, Tindle HA, Tyrfingsson T, Wall TL, Weir DR, Weisner C, Whitfield JB, Winsvold BS, Yin J, Zuccolo L, Bierut LJ, Hveem K, Lee JJ, Munafò MR, Saccone NL, Willer CJ, Cornelis MC, David SP, Hinds DA, Jorgenson E, Kaprio J, Stitzel JA, Stefansson K, Thorgeirsson TE, Abecasis G, Liu DJ and Vrieze S. Association studies of up to 1.2 million individuals yield new insights into the genetic etiology of tobacco and alcohol use. *Nature genetics*. 2019;51:237-244.

11. Hoggart CJ, Clark TG, De Iorio M, Whittaker JC and Balding DJ. Genome-wide significance for dense SNP and resequencing data. *Genet Epidemiol*. 2008;32:179-85.

12. The Genotype-Tissue Expression (GTEx) project. *Nat Genet*. 2013;45:580-5.

13. Thompson A, Cook J, Choquet H, Jorgenson E, Yin J, Kinnunen T, Barclay J, Morris AP and Pirmohamed M. Functional validity, role, and implications of heavy alcohol consumption genetic loci. *Science advances*. 2020;6:eaay5034.

14. Pandey P, Singh A, Kaur H, Ghosh-Roy A and Babu K. Increased dopaminergic neurotransmission results in ethanol dependent sedative behaviors in Caenorhabditis elegans. *PLoS Genet*. 2021;17:e1009346.
15. Alaimo JT, Davis SJ, Song SS, Burnette CR, Grotewiel M, Shelton KL, Pierce-Shimomura JT, Davies AG and Bettinger JC. Ethanol metabolism and osmolarity modify behavioral responses to ethanol in C. elegans. *Alcohol Clin Exp Res*. 2012;36:1840-50.
16. Diegelmann S, Jansen A, Jois S, Kastenholz K, Velo Escarcena L, Strudthoff N and Scholz H. The CApillary FEeder Assay Measures Food Intake in Drosophila melanogaster. *Journal of visualized experiments : JoVE*. 2017.
17. Aleyakpo B, Umukoro O, Kavlie R, Ranson DC, Thompsett A, Corcoran O and Casalotti SO. G-protein αq gene expression plays a role in alcohol tolerance in Drosophila melanogaster. *Brain and neuroscience advances*. 2019;3:2398212819883081.
18. Tennessen JM, Barry WE, Cox J and Thummel CS. Methods for studying metabolism in Drosophila. *Methods*. 2014;68:105-15.
19. Johnson JR, Rajamanoharan D, McCue HV, Rankin K and Barclay JW. Small Heat Shock Proteins Are Novel Common Determinants of Alcohol and Nicotine Sensitivity in Caenorhabditis elegans. *Genetics*. 2016;202:1013-27.
20. Kamath RS and Ahringer J. Genome-wide RNAi screening in Caenorhabditis elegans. *Methods*. 2003;30:313-21.
21. Rual JF, Ceron J, Koreth J, Hao T, Nicot AS, Hirozane-Kishikawa T, Vandenhaute J, Orkin SH, Hill DE, van den Heuvel S and Vidal M. Toward improving Caenorhabditis elegans phenome mapping with an ORFeome-based RNAi library. *Genome Res*. 2004;14:2162-8.
22. Davies AG, Pierce-Shimomura JT, Kim H, VanHoven MK, Thiele TR, Bonci A, Bargmann CI and McIntire SL. A central role of the BK potassium channel in behavioral responses to ethanol in C. elegans. *Cell*. 2003;115:655-66.
23. Canela-Xandri O, Rawlik K and Tenesa A. An atlas of genetic associations in UK Biobank. *Nature Genetics*. 2018;50:1593-1599.
24. Castagné R, Boulangé CL, Karaman I, Campanella G, Santos Ferreira DL, Kaluarachchi MR, Lehne B, Moayyeri A, Lewis MR, Spagou K, Dona AC, Evangelos V, Tracy R, Greenland P, Lindon JC, Herrington D, Ebbels TMD, Elliott P, Tzoulaki I and Chadeau-Hyam M. Improving Visualization and Interpretation of Metabolome-Wide Association Studies: An Application in a Population-Based Cohort Using Untargeted 1H NMR Metabolic Profiling. *Journal of proteome research*. 2017;16:3623-3633.
25. Chadeau-Hyam M, Ebbels TMD, Brown IJ, Chan Q, Stamler J, Huang CC, Daviglus ML, Ueshima H, Zhao L, Holmes E, Nicholson JK, Elliott P and De Iorio M. Metabolic Profiling and the Metabolome-Wide Association Study: Significance Level For Biomarker Identification. *Journal of proteome research*. 2010;9:4620-4627.
26. Chong J, Soufan O, Li C, Caraus I, Li S, Bourque G, Wishart DS and Xia J. MetaboAnalyst 4.0: towards more transparent and integrative metabolomics analysis. *Nucleic Acids Research*. 2018;46:W486-W494.
27. Corl AB, Berger KH, Ophir-Shohat G, Gesch J, Simms JA, Bartlett SE and Heberlein U. Happyhour, a Ste20 family kinase, implicates EGFR signaling in ethanol-induced behaviors. *Cell*. 2009;137:949-60.
28. Martin GG, Danneberg H, Kumar LS, Atshaves BP, Erol E, Bader M, Schroeder F and Binas B. Decreased liver fatty acid binding capacity and altered liver lipid distribution in

mice lacking the liver fatty acid-binding protein gene. *The Journal of biological chemistry*. 2003;278:21429-38.
29. Taneja-Bageshwar S and Gumienny TL. Regulation of TGFβ superfamily signaling by two separable domains of glypican LON-2 in C. elegans. *Worm*. 2013;2:e23843.
30. Zhao J, Hu L, Gui W, Xiao L, Wang W, Xia J, Fan H, Li Z, Zhu Q, Hou X, Chu H, Seki E and Yang L. Hepatocyte TGF-β Signaling Inhibiting WAT Browning to Promote NAFLD and Obesity Is Associated With Let-7b-5p. *Hepatology communications*. 2022;6:1301-1321.
31. Xiang X, Ohshiro K, Zaidi S, Yang X, Bhowmick K, Vegesna AK, Bernstein D, Crawford JM, Mishra B, Latham PS, Gough NR, Rao S and Mishra L. Impaired reciprocal regulation between SIRT6 and TGF-β signaling in fatty liver. *FASEB journal : official publication of the Federation of American Societies for Experimental Biology*. 2022;36:e22335.
32. Ciuclan L, Ehnert S, Ilkavets I, Weng HL, Gaitantzi H, Tsukamoto H, Ueberham E, Meindl-Beinker NM, Singer MV, Breitkopf K and Dooley S. TGF-beta enhances alcohol dependent hepatocyte damage via down-regulation of alcohol dehydrogenase I. *J Hepatol*. 2010;52:407-16.
33. Boucard AA, Maxeiner S and Südhof TC. Latrophilins function as heterophilic cell-adhesion molecules by binding to teneurins: regulation by alternative splicing. *The Journal of biological chemistry*. 2014;289:387-402.
34. Tews D, Schwar V, Scheithauer M, Weber T, Fromme T, Klingenspor M, Barth TF, Möller P, Holzmann K, Debatin KM, Fischer-Posovszky P and Wabitsch M. Comparative gene array analysis of progenitor cells from human paired deep neck and subcutaneous adipose tissue. *Molecular and cellular endocrinology*. 2014;395:41-50.
35. Tews D, Fromme T, Keuper M, Hofmann SM, Debatin KM, Klingenspor M, Wabitsch M and Fischer-Posovszky P. Teneurin-2 (TENM2) deficiency induces UCP1 expression in differentiating human fat cells. *Molecular and cellular endocrinology*. 2017;443:106-113.
36. Scannell Bryan M, Argos M, Pierce B, Tong L, Rakibuz-Zaman M, Ahmed A, Rahman M, Islam T, Yunus M, Parvez F, Roy S, Jasmine F, Baron JA, Kibriya MG and Ahsan H. Genome-Wide Association Studies and Heritability Estimates of Body Mass Index Related Phenotypes in Bangladeshi Adults. *PloS one*. 2014;9:e105062.
37. Elsworth B, Lyon M, Alexander T, Liu Y, Matthews P, Hallett J, Bates P, Palmer T, Haberland V, Smith GD, Zheng J, Haycock P, Gaunt TR and Hemani G. The MRC IEU OpenGWAS data infrastructure. *bioRxiv*. 2020:2020.08.10.244293.

Figure legends

Figure 1: Association of alcohol consumption with circulating metabolites in the Airwave study. Pie chart illustrates percentages of metabolite classes (RefMet "Super class") present that are significantly associated with alcohol consumption.

Figure 2: Analysis of alcohol intake and sedation in adult flies. (A) Mapping *Drosophila* orthologues of human genes involved in ethanol consumption. (B) Analysis of ethanol intake (left column) and sedation (right column) in the Drosophila RNAi lines. The numerical values show the difference between means (RNAi - Control). Values in parenthesis are the number of biological replicates for respectively, the control and the RNAi line. (C) Analysis of TAG levels in adult flies fed either with normal or ethanol-containing food (mean ± standard error of mean; asterisks, 2-way ANOVA with Tukey's multiple comparisons test). The number of biological replicates per experimental variable (n) is indicated in either the respective figure or figure legend. No sample was excluded from the analysis unless otherwise stated. Blinding was not performed. Normality was assessed before deciding on which parametric or non-parametric test to use for inferential statistics. Statistical significance is indicated as * for $P < 0.05$, ** for $P < 0.01$, *** for $P < 0.001$, **** for $P < 0.0001$ and NS for $P \geq 0.05$.

Figure 3: Quantification of alcohol phenotypes for *C. elegans* genes. (A) Nematodes with *loss-of-function* mutations in worm orthologues of *ACTR1B* (*arp*-1), *ARPC1B* (*arx-3*), *GPN1* (*gop-2*), *MLXIPL* (*mml-1*), *STAT6* (*sta-1*), *TENM2* (*ten-1*), *KIF26A* (*vab-8*), *SLC4A8* (*abts-1*), *MAPT* (*ptl-1*) and *SCN8A* (cca-1) were acutely exposed to ethanol and the resultant effect on rate of locomotion (thrashes per minute) was quantified. In comparison with Bristol N2 wild-type worms, significant differences were identified for *arp-1*, *arx-3* and *ptl-1*. Data is presented normalized to locomotion rate of untreated worms. *P<0.01. (B) RNAi confirmation of positively identified genes involved in alcohol phenotypes. In comparison to controls, RNAi knockdown of worm orthologues of *ACTR1B* (*arp*-1) and *MAPT* (*ptl-1*) phenocopied the *loss-of-function* mutations, whereas *ARPC1B* (*arx-3*) knockdown had no effect. *P<0.01. n.s., not significant.

Supplementary Figure S1: Basal locomotion rates. (A) Nematodes with *loss-of-function* mutations in worm orthologues of *ACTR1B* (*arp*-1), *ARPC1B* (*arx-3*), *GPN1* (*gop-2*), *MLXIPL* (*mml-1*), *STAT6* (*sta-1*), *TENM2* (*ten-1*), *KIF26A* (*vab-8*), *SLC4A8* (*abts-1*), *MAPT* (*ptl-1*) and *SCN8A* (cca-1) were quantified for locomotion rate (thrashes per minute). In comparison with Bristol N2 wild-type worms, significant differences were identified for *ten-1*, *vab-8*, *abts-1* and *cca-1*. *P<0.01. #P<0.05. (B) Quantification of locomotion rate for worms subjected to RNAi knockdown. In comparison to controls, RNAi knockdown of *arp-1*, *arx-3* or *ptl-1* had no effect on basal locomotion rate.

Table 1- Overview of the genetic variants with effect on alcohol consumption, liver enzymes and gene expression.

Alcohol SNP *	Annotated Gene	eQTL tissue	eQTL effect size§	eQTL P value†	*Drosophila* ortholog‡	FlyBase score out of 15§	Wormbase: *C.elegans* ortholog (score)‡	*Drosophila* strains	*C. elegans* strain
rs823114	*NUCKS1*	Thyroid	-0.16	5.80×10^{-18}	-	-	-	-	-
rs1260326	*GCKR*	Thyroid	0.22	1.80×10^{-08}	-	-	-	-	-
rs2178197	*GPN1*	Brain-Cerebellar Hemisphere	-0.31	6.50×10^{-08}	*cg3704*	13	*gop-2 (9)* ¶	*CG3704* (55294)	RG5036 *gop-2 (gk5528)*,
rs13032049	*WDPCP*	Adipose-Subcutaneous	-0.16	0.0003	*frtz*	13	-	*Frz* (55649)	-
rs11692435	*ACTR1B*	Thyroid	0.89	1.50×10^{-80}	*arp1*	12	*arp-1 (8)* ¶	Lethal, not studied	FX4735 *arp-1 (tm4735)*
rs12646808	*MSANTD1*	Thyroid	-0.25	1.30×10^{-09}	*cg18766*	8	-	-	-
rs11940694	*KLB*	Liver	0.22	4.00×10^{-05}	*cg9701*	6	*klo-1 (5), klo-2 (5)*	-	**Not studied**
rs1229984	*ADH1B*	Esophagus-Gastroesophageal Junction	-1.4	1.90×10^{-16}	*fdh*	7	*adh-5 (5)*	-	**Not studied**
rs10078588	*TENM2*	Thyroid	0.29	2.80×10^{-15}	*ten-m*	13	*ten-1 (10)* ¶	*TenM* (29390)	VC518 *ten-1 (ok641)*,
rs34060476	*MLXIPL*	Pancreas	0.51	4.50×10^{-18}	*mondo*	12	*mml-1 (9)*	*Mondo* (27059)	RB954 *mml-1 (ok849*)
rs10249167	*ARPC1B*	Thyroid	0.3	1.80×10^{-25}	*arpc1*	13	*arx-3 (10)* ¶	*Arpc1* (31246)	VC3166 *arx-3 (ok1122)*
rs988748	*BDNF*	Brain - Nucleus accumbens basal ganglia	0.22	9.70×10^{-06}	-	-	-	-	-
rs2071305	*MYBPC3*	Whole blood	-0.13	2.30×10^{-11}	*hbs*	1	-	-	-
rs7121986	***DRD2***	Esophagus - Muscularis	0.26	3.50×10^{-08}	*dop2r*	9	*dop-2 (6), dop-3 (6)*	-	**Not studied**
rs10876188	*SLC4A8*	Cells-Cultured fibroblasts	-0.12	1.80×10^{-07}	*ndae1*	12	*abts-1 (9)*	Lethal, not studied	RB1381 *abts-1 (ok1566)*
rs7958704	*SCN8A*	Nerve - Tibial	-0.25	2.90×10^{-10}	*para*	12	*cca-1 (1), unc-77 (1), egl-19 (4)*	*Para* (33923)	JD21 *cca-1 (ad1650)*

rs12312693	*STAT6*	Brain - Frontal Cortex BA9	0.23	8.00×10^{-05}	*stat92e*	9	*sta-2 (4), sta-1 (7)*	-	RB796 *sta-1 (ok587)*
rs11625650	*KIF26A*	Whole blood	-0.26	9.10×10^{-12}	*cg14535*	7	*vab-8 (2)*	-	NG2484 *vab-8 (gm84)*
rs1421085	*FTO*	Muscle - Skeletal	0.14	7.50×10^{-08}	-	-	-	-	-
rs11648570	***PMFBP1***	Esophagus - Mucosa	-0.29	9.00×10^{-05}	*cg12702*	1	-	-	-
rs3803800	*TNFSF13*	Brain - Cortex	-0.2	2.50×10^{-08}	*egr*	3	-	-	-
rs1053651	*TCAP*	Muscle - Skeletal	-0.11	4.90×10^{-11}	-	-	-	-	-
rs1991556	*MAPT*	Brain - Cerebellum	-0.28	1.80×10^{-06}	*tau*	7	*ptl-1 (8)*	-	RB809 *ptl-1 (ok621)*
rs4815364	*ACSS1*	Brain - Cerebellar Hemisphere	-0.47	1.70×10^{-12}	*accoas*	6	-	-	-

* Alcohol SNPs associated with liver enzymes at replication P value <0.000476. Liver enzyme GWAS summary statistics obtained from Pazoki et al 2021.
† Normalised effect size and P-value obtained from GTEx portal. ‡ Orthologs were extracted from FlyBase and WormBase. § number of FlyBase tools that support the gene-pair relationship out of a total number of tools (n=15) that computed relationships between *Homo sapiens* and *Drosophila melanogaster*.
¶ Genes for which mutations were homozygous lethal and as such, heterozygote mutations balanced by chromosomal translocations were instead analysed.
eQTL: expression quantitative trait loci.

Table 2- Overview of the causal effect of alcohol on circulating metabolites using the inverse variance weighted two-sample Mendelian randomisation multiple instrument method.

Metabolite	Name	Main class	Beta (95% CI)	P-value
SLPOS_457.3339_0.7190	CAR DC18:1	Fatty esters	-1.13 (-2.14, -0.11)	0.036
SLPOS_579.5352_8.2395	DG 34:0	Diradylglycerols	0.91 (0.05,1.77)	0.047
SLPOS_606.5548_8.3326	DG 36:1	Diradylglycerols	0.92 (0.07,1.77)	0.041
SLPOS_745.5603_5.7424	PC 33:2	Glycerophosphocholines	0.78 (0.07,1.5)	0.040
SLPOS_629.5424_6.9217	PE 38:4	Glycerophosphoethanolamines	1.2 (0.15,2.25)	0.032
SHPOS_625.5174_2.8914	PE 38:5	Glycerophosphoethanolamines	1.1 (0.1,2.1)	0.039
SLPOS_201.0516_0.6580	Piperine	Alkaloids	1.16 (0.1,2.22)	0.040
SHPOS_807.6348_4.4974	SM 40:2;O2	Sphingomyelins	1.06 (0.15,1.96)	0.028
SHPOS_827.7093_0.5837	TG 48:1	Triradylglycerols	0.95 (0.13,1.77)	0.030
SLPOS_898.7965_11.2257	TG 53:1	Triradylglycerols	0.93 (0.1,1.75)	0.036
SLPOS_888.8079_10.7574	TG 53:3	Triradylglycerols	0.96 (0.06,1.85)	0.045
SLPOS_607.5599_11.1277	TG 54:2	Triradylglycerols	0.95 (0.19,1.71)	0.019
SLPOS_1027.7392_10.9051	TG 54:3	Triradylglycerols	1.06 (0.21,1.91)	0.020
SLPOS_984.8508_11.7812	TG 58:1	Triradylglycerols	1.12 (0.44,1.81)	0.003
SLPOS_687.6307_11.5996	TG 58:2	Triradylglycerols	1.08 (0.27,1.9)	0.014
SLPOS_689.6461_11.7895	TG 60:2	Triradylglycerols	1.24 (0.52,1.95)	0.002

CI, Confidence Interval. Effect estimates and 95% CI is given for the inverse variance weighted method of Mendelian randomisation.

Table 3 - Overview of the significant associations between SNPs in *WDPCP*, *TENM2*, and *GPN1* with Phewas traits within the UK Biobank Edinburg Gene Atlas.

Trait	rs10078588-A (*TENM2*)			rs13032049-G (*WDPCP*)			rs2178197-G (*GPN1*)		
	Beta	Z value	*P*-value	Beta	Z value	*P*-value	Beta	Z value	*P*-value
Bread intake	**0.0940**	**5.6599**	**7.57×10^{-09}**	-0.0413	1.9952	0.023013	0.0113	-0.0152	0.49393
Alcohol weekly intake frequency (high to low)	**-0.0151**	**5.2063**	**9.63×10^{-08}**	-0.0068	1.8512	0.032067	-0.0060	-1.7782	0.037686
Oily fish intake	**-0.0084**	**4.5347**	**2.88×10^{-06}**	-0.0045	1.9777	0.023981	-0.0023	-0.8290	0.20354
Beef intake	**0.0076**	**4.4475**	**4.34×10^{-06}**	0.0001	-2.0097	0.97777	-0.0046	-2.5069	0.0060897
Fresh fruit intake	**-0.0134**	**4.4423**	**4.45×10^{-06}**	**-0.0126**	**3.6997**	**0.00010792**	0.0062	-1.8056	0.035493
Water intake	**0.0175**	**3.9575**	**3.79×10^{-05}**	0.0013	-0.7977	0.78748	0.0002	1.7862	0.96297
Hot drink temperature	**-0.0044**	**3.7732**	**8.06×10^{-05}**	-0.0025	1.6849	0.046005	-0.0005	0.3865	0.65045
Salt added to food	**0.0065**	**3.7333**	**9.45×10^{-05}**	**0.0095**	**5.0066**	**2.77×10^{-07}**	0.0013	-0.1656	0.43425
Pork intake	**0.0055**	**3.6391**	**0.00013679**	0.0027	1.3625	0.086524	-0.0025	-1.3401	0.090113
Trunk fat percentage	**-0.0460**	**3.5231**	**0.0002133**	-0.0212	1.1416	0.12681	-0.0036	0.7640	0.77756
Current tobacco smoking	**0.0040**	**3.4565**	**0.00027362**	0.0025	1.7048	0.044118	0.0009	-0.2750	0.39164
Coffee intake	**0.0150**	**3.4345**	**0.00029683**	**-0.0153**	**3.1112**	**0.00093177**	-0.0049	-0.6910	0.24479
Smoking status	0.0034	2.4182	0.007799	**0.0061**	**4.1218**	**1.88×10^{-05}**	0.0008	0.1444	0.5574
Body mass index (BMI)	-0.0207	2.3121	0.010387	**-0.0299**	**3.1017**	**0.00096205**	-0.0224	-2.5132	0.0059828
Frequency of solarium/sunlamp use	0.0184	1.6438	0.050112	**0.0353**	**3.1830**	**0.00072886**	-0.0078	-0.2305	0.40884
I20 Angina pectoris	-0.0006	1.0920	0.13741	**-0.0016**	**3.3388**	**0.00042077**	-0.0012	-2.6478	0.004051
heart attack/myocardial infarction	-0.0003	0.4272	0.33463	**-0.0012**	**3.2628**	**0.00055166**	-0.0005	-1.2536	0.105
depression	-0.0004	0.2607	0.39716	**0.0019**	**3.2659**	**0.0005456**	0.0005	-0.3721	0.3549
Comparative body size at age 10	0.0009	0.0955	0.46197	**-0.0058**	**3.9702**	**3.59×10^{-05}**	-0.0005	0.5357	0.7039
hypertension	-0.0027	3.1727	0.00075503	-0.0004	-0.3906	0.65197	**-0.0036**	**-4.2605**	**1.02×10^{-05}**
I10 Essential (primary) hypertension	-0.0018	2.1221	0.016914	-0.0007	0.2099	0.41689	**-0.0029**	**-3.7581**	**8.56×10^{-05}**
I10-I15 Hypertensive diseases	-0.0018	2.1187	0.01706	-0.0007	0.3274	0.37167	**-0.0029**	**-3.7891**	**7.56×10^{-05}**
K57 Diverticular disease of intestine	0.0010	1.6594	0.048518	0.0011	1.7320	0.041639	**-0.0020**	**-3.8683**	**5.48×10^{-05}**
Neutrophil count	0.0049	1.6565	0.048807	-0.0045	1.2254	0.11022	**-0.0105**	**-3.9811**	**3.43×10^{-05}**
Mean sphered cell volume	0.0113	0.8969	0.18489	0.0033	-0.6078	0.72835	**0.0443**	**-4.9818**	**3.15×10^{-07}**

Red blood cell (erythrocyte) count	-0.0007	0.7191	0.23603	0.0005	0.0232	0.49073	**-0.0033**	**-5.4067**	**3.21×10^{-08}**
Number of treatments/medications taken	-0.0051	0.5243	0.30005	0.0061	0.6081	0.27156	**-0.0240**	**-4.6438**	**1.71×10^{-06}**
Monocyte percentage	0.0034	0.3546	0.36145	0.0092	1.8981	0.028842	**0.0195**	**-4.9676**	**3.39×10^{-07}**
Number of operations, self-reported	-0.0023	0.1247	0.4504	0.0078	2.0778	0.018863	**-0.0135**	**-4.3264**	**7.58×10^{-06}**
Impedance of arm (left)	-0.0490	0.1147	0.45435	0.1348	1.5035	0.066356	**0.2789**	**-4.0241**	**2.86×10^{-05}**
Platelet count	0.0618	0.0081	0.49676	-0.2315	1.9989	0.02281	**-0.3725**	**-3.8710**	**5.42×10^{-05}**
Impedance of arm (right)	-0.0342	-0.2218	0.58775	0.1734	2.1919	0.014193	**0.2712**	**-4.0691**	**2.36×10^{-05}**
Haemoglobin concentration	0.0009	-0.2824	0.61117	0.0035	1.4670	0.071193	**-0.0070**	**-3.8314**	**6.37×10^{-05}**
Platelet crit	0.0000	-0.3210	0.62591	-0.0002	1.9033	0.028499	**-0.0003**	**-3.6987**	**0.00010837**
Mean reticulocyte volume	0.0050	-0.5194	0.69827	-0.0033	-0.9130	0.81939	**0.0601**	**-4.3903**	**5.66×10^{-06}**
High light scatter reticulocyte percentage	0.0001	-0.7321	0.76795	-0.0001	-0.9971	0.84065	**-0.0016**	**-4.3662**	**6.32×10^{-06}**
Reticulocyte count	0.0000	-0.7929	0.78608	0.0000	-1.7136	0.9567	**-0.0002**	**-5.1461**	**1.33×10^{-07}**
Impedance of whole body	0.0293	-0.8067	0.79009	0.3281	2.4133	0.0079041	**0.4599**	**-3.9397**	**4.08×10^{-05}**
Reticulocyte percentage	0.0002	-0.9360	0.82536	-0.0002	-1.0798	0.85989	**-0.0042**	**-4.0940**	**2.12×10^{-05}**
prostate problem (not cancer)	-0.0001	-0.9578	0.83092	-0.0004	0.0190	0.49243	**-0.0021**	**-3.4902**	**0.00024137**
Lymphocyte percentage	0.0024	-1.0500	0.85313	0.0327	1.9647	0.024727	**0.0612**	**-4.4883**	**3.59×10^{-06}**
Neutrophil percentage	-0.0026	-1.0949	0.86321	-0.0299	1.4172	0.078209	**-0.0712**	**-4.4756**	**3.81×10^{-06}**
High light scatter reticulocyte count	0.0000	-1.4700	0.92922	0.0000	-1.3850	0.91698	**-0.0001**	**-5.3912**	**3.50×10^{-08}**
Haematocrit percentage	0.0004	-1.4994	0.93312	0.0100	1.4070	0.079718	**-0.0207**	**-3.8409**	**6.13×10^{-05}**
other urological problem (male)	0.0000	-2.7944	0.9974	0.0000	-1.5853	0.94355	**-0.0022**	**-3.4515**	**0.00027871**

Significant associations are depicted in bold.

Table 4- Overview of the association of SNPs in *WDPCP*, *TENM2*, and *GPN1* with disorders of lipoprotein metabolism and other lipidaemias (ICD code E78) within the UK Biobank Edinburgh Gene Atlas.

SNP	chromosome	Base pair location	Effect allele	Beta (effect estimate)	95% CI lower bound	95% CI upper bound	P-value	MAF	HWE
rs10078588	5 (*TENM2)*	166816176	A	-0.00134	-2.49×10^{-04}	-2.44×10^{-03}	0.016	0.474444	0.524
rs13032049	2 (*WDPCP*)	63581507	G	-0.00138	-1.59^{-04}	-2.60×10^{-03}	0.027	0.278928	0.5096
rs2178197	2 (*GPN1*)	27860551	G	-0.00144	-3.36×10^{-04}	-2.55×10^{-03}	0.011	0.424097	0.2078

Results obtained from the Edinburgh Gene Atlas. CI, Confidence Interval; SNP, Single Nucleotide Polymorphism; MAF, minor allele frequency; HWE, Hardy Weinberg Equilibrium.

Table 5- Overview of the significant results from inverse variance weighted Mendelian randomisation analysis for the effect of gene expression of ENSG00000115507 on liver traits using the MRC IEU OpenGWAS data infrastructure [37].

Liver trait	Number of SNPs	Effect Estimate	Standard Error	*P* value
Fatty acid-binding protein, liver	6	-0.1053	0.0554	0.0576
Liver enzyme levels (alanine transaminase)	6	-0.0017	0.0014	0.2355
Fibrosis and cirrhosis of liver	6	-0.1972	0.0967	0.0415
Liver & bile duct cancer	6	0.0003	0.0001	0.0284

Figure 1-

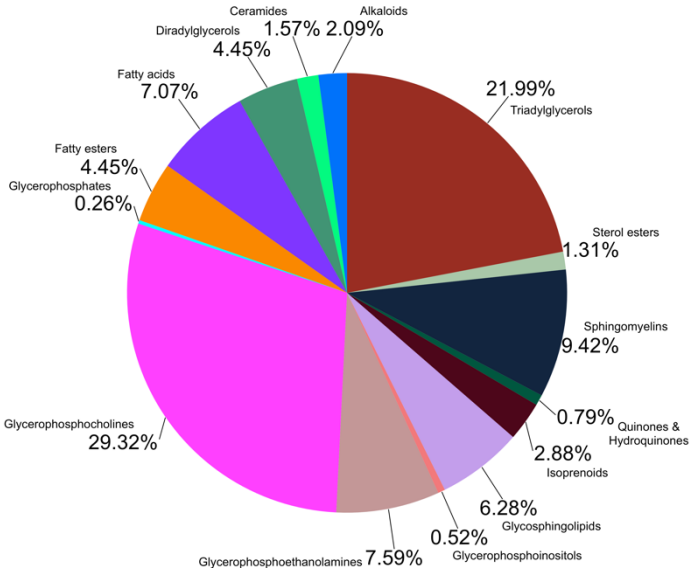
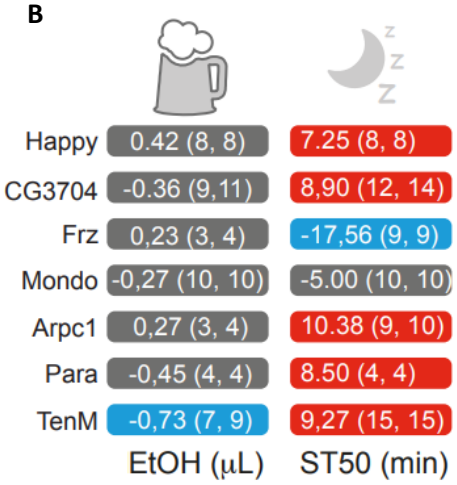


Figure 2-

A

Alcohol SNP	Gene	Orthologue (*Drosophila*)	True Orthologue
rs1278197	*GPN1*	*CG3704*	Yes
rs13032049	*WDPCP*	*frtz*	Yes
rs34060476	*MLXIPL*	*Mondo*	49%
rs10249167	*ARPC1B*	*Arpc1*	50%
rs7958704	*SCN8A*	*para*	46%
rs10078588	*TENM2*	*Ten-m*	Yes

B



C

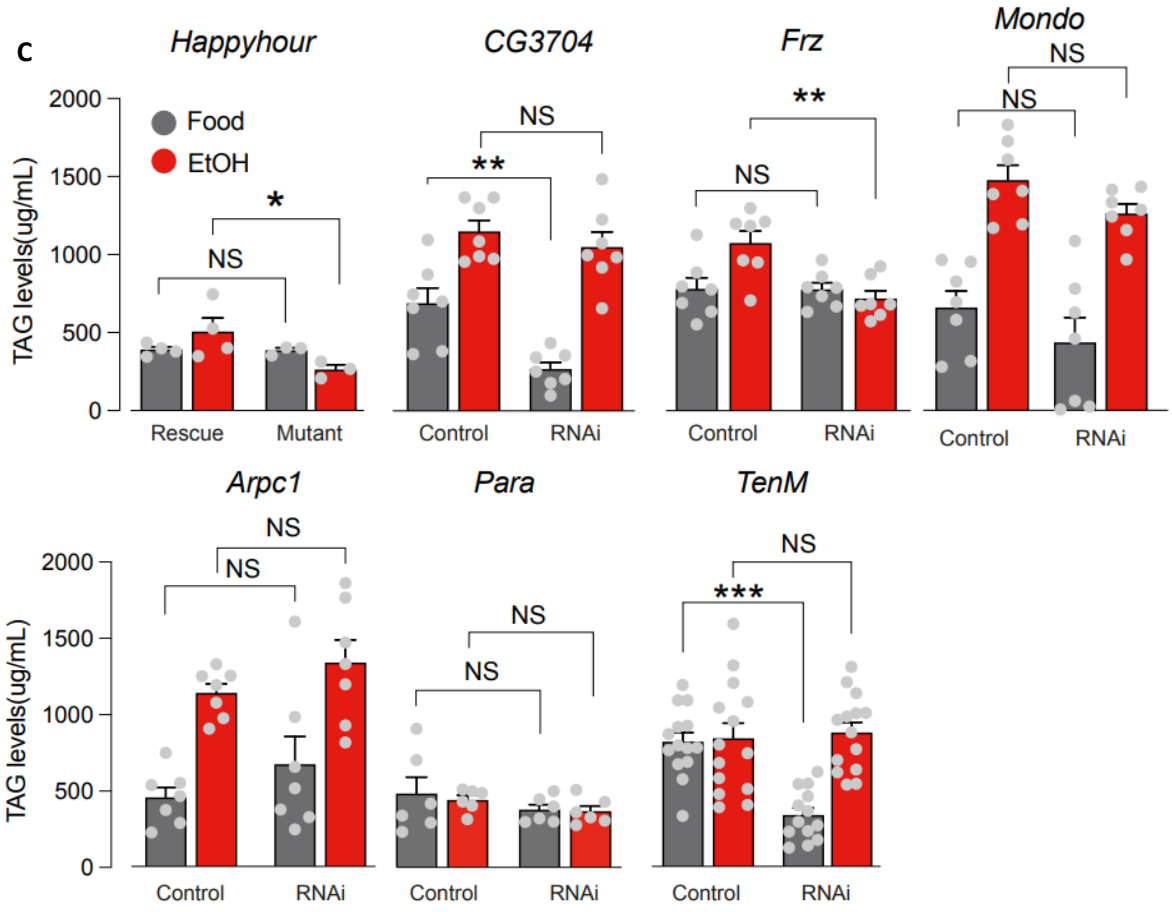
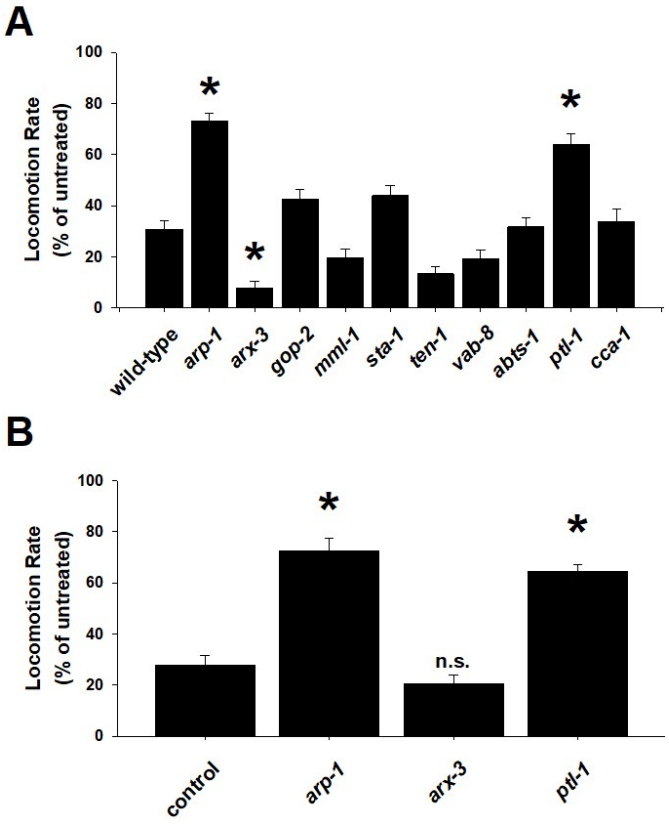


Figure 3-



Supplementary Figure S1-