The Role of the Aryl Hydrocarbon Receptor Polymorphisms in Modulating 2,3,7,8-tetrachlorodibenzo-ρ-dioxin (TCDD)-induced HMGCR expression

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Introduction

- The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor that regulates gene expression of a battery of genes important for detoxification and metabolism[1]
- Ligands that activate the AHR include halogenated planar aromatic hydrocarbons. The most potent of which is 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD)[1]
- In the body cholesterol is either obtained through the diet or is synthesized in the liver via the mevalonate pathway[2]
- 3-hydroxy-3-methlglutaryl coenzyme A reductase (HMGCR) is the rate-limiting enzyme of the cholesterol synthesis pathway[2]
- Previous studies have shown a repression of genes involved in cholesterol biosynthesis, including HMGCR, after AHR activation[2]
- A genome-wide association study (GWAS) aims to correlate genetic variations to specific traits
- A GWAS using data from the UK Biobank Mendelian Trait identified polymorphisms around the AHR locus that were correlated with reduced cholesterol levels

Methods

Quick Change Site-Directed Mutagenesis: The protocol for Quick Change SDM was provided courtesy of Dr. David Arnosti, Michigan State University. AHR wild type and AHR cSNP P275K were amplified at 1Kb/min using custom oligonucleotide primers in a standard thermocycler. Annealing temperature was 64°C for 60 seconds.

Bacterial Transformation: For AHR containing cSNP at R554L and R554L/P275K, OneShot Stbl3 chemically competent E. Coli were thawed on ice for 30 minutes, then heat shocked for 45 seconds at 42°C. Each vial was shaken in incubator at 37°C for 45 minutes and spread on ampicillin plates.

Results

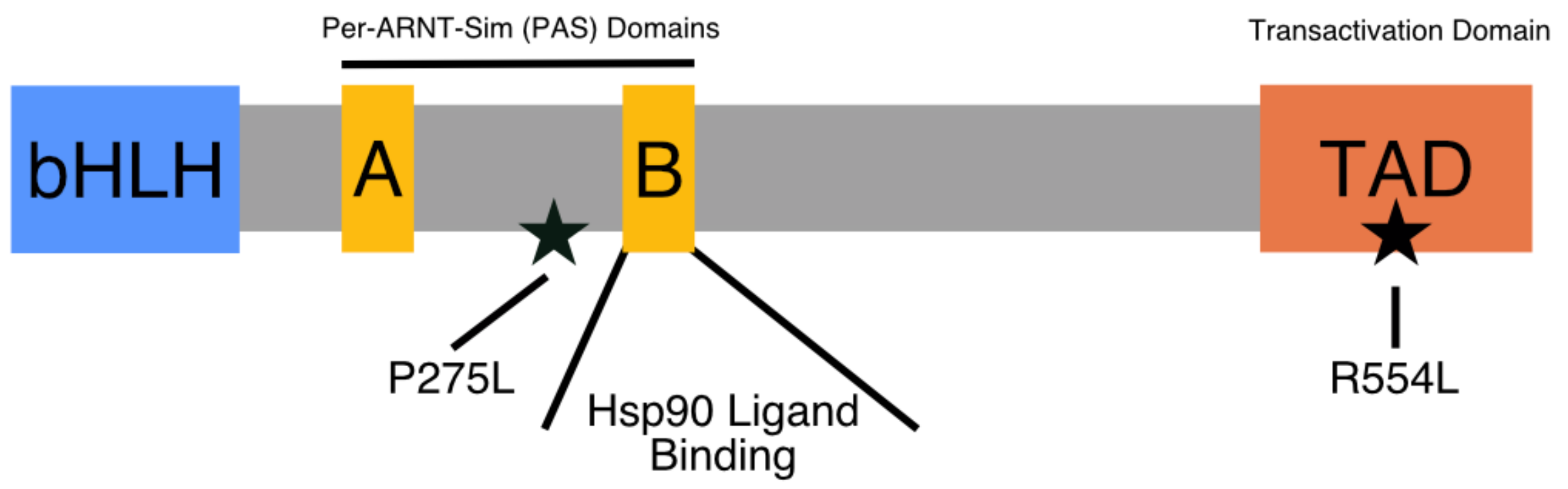

Figure 1: Structure of the Aryl Hydrocarbon Receptor. The Aryl Hydrocarbon Receptor (AHR) is a multidomain protein with several important domains including the basic helix-loop-helix (bHLH), a Per-ARNT-Sim (PAS) domain divided into two, A and B, as well as the transactivation domain (TAD). The bHLH and PAS domains contribute to heterodimerization. Ligand binding occurs at the PAS B domain.

Results

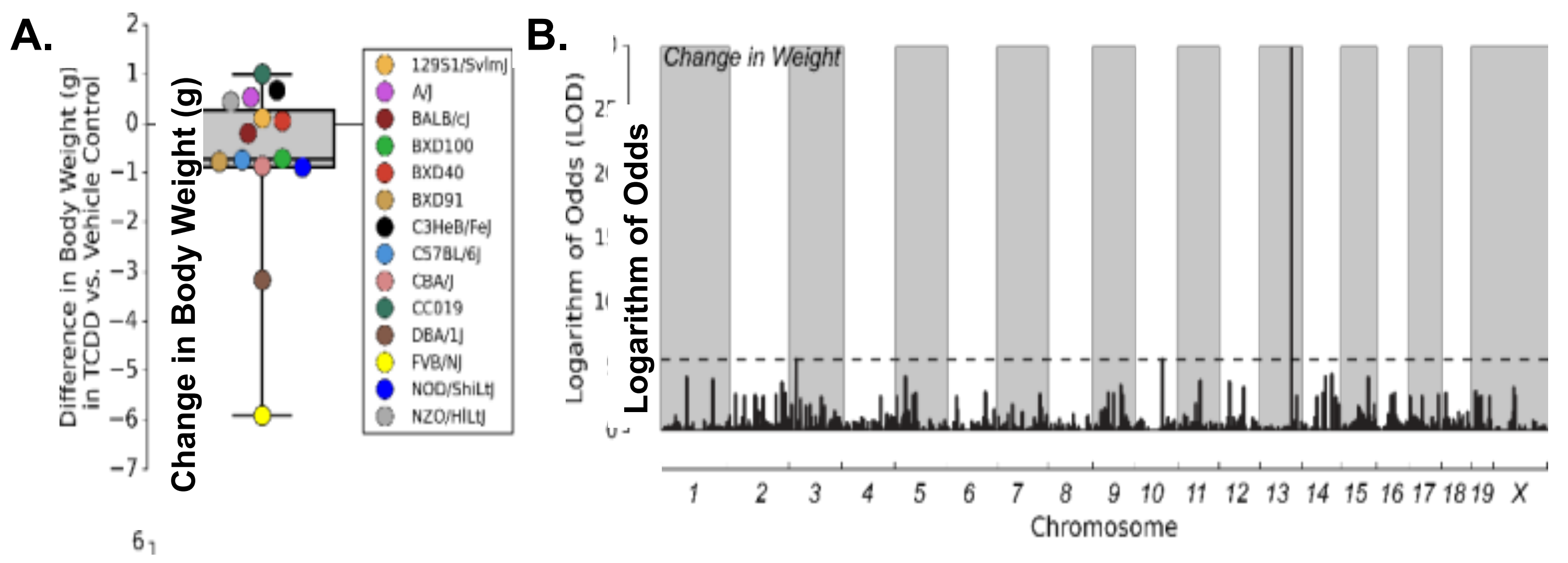

Figure 2 QTL analysis of inter-strain variability in TCDD-induced change in body weight. **C**hange in average weight (g) (**A**) induced by 100 ng/kg/day TCDD in 14 genetically-diverse strains. QTL analysis identified an association between Chromosome 13 and weight change (p<0.001). (**B**). Dotted line indicates threshold of genome-wide significance determined with permutation testing (n=10,000).

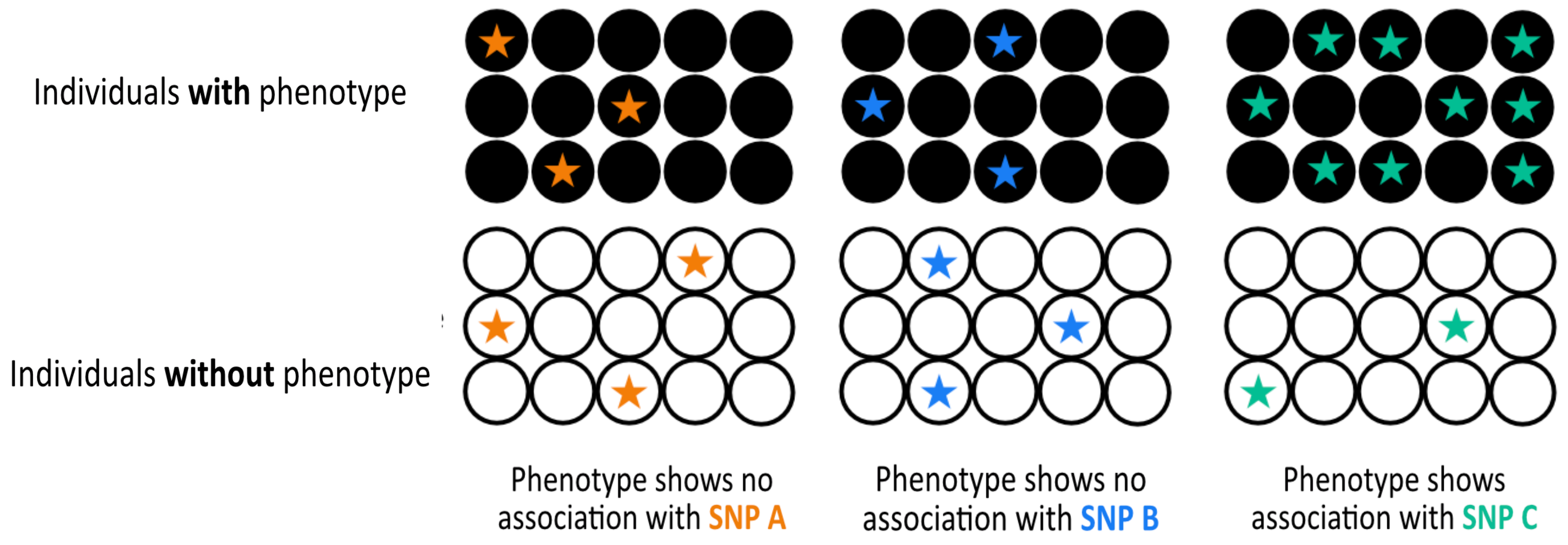

Figure 3: Generic Example of Genome-Wide Association Study. Black circles represent individuals who possess phenotype of interest, white circles represent those who don't. Colored stars represent presence of certain single nucleotide polymorphism (SNP) within their genome. In this example, a correlation between SNP C and the phenotype of interest is observed.

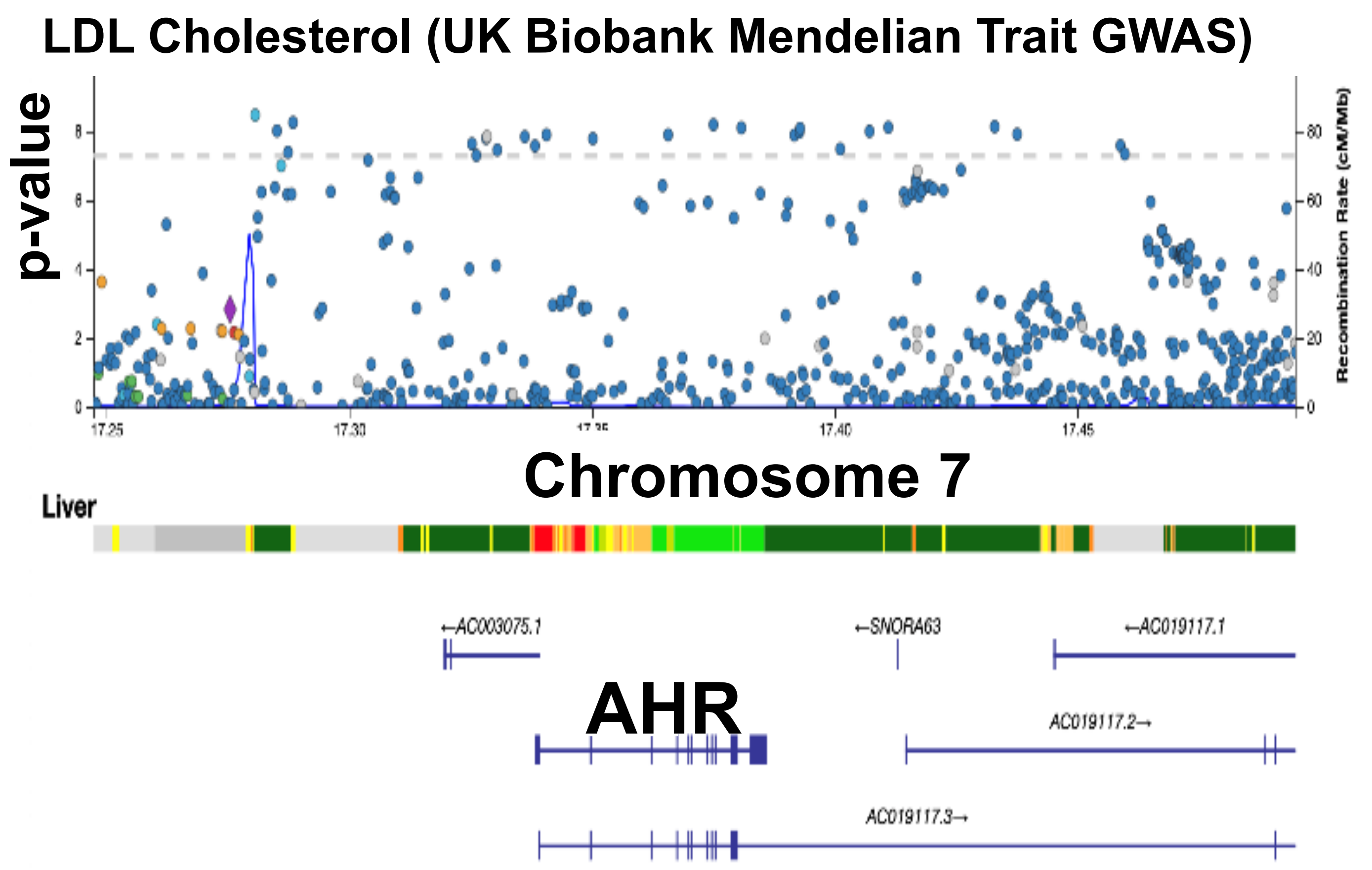

Figure. 4 Manhattan Plot of AHR variants associated with human LDL. The UK Biobank Mendelian Trait GWAS database was used to show that a large number of AHR variants correlated with reduced LDL levels near the AHR locus.

Results

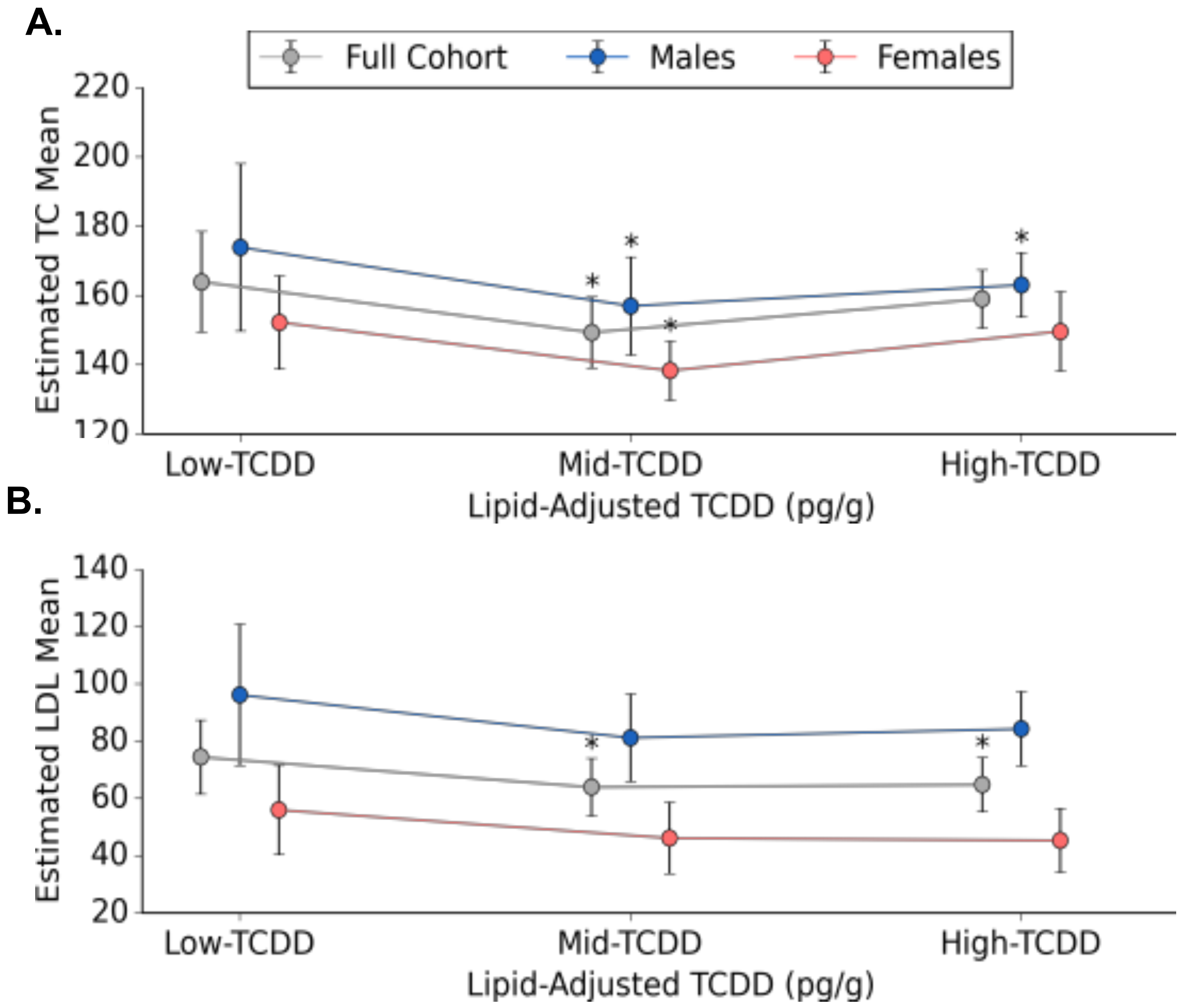

Figure. 5 Human Relevance of the Impact of TCDD on Cholesterol. NHANES data were used to correlate serum TCDD levels with serum total cholesterol (TC) (**A**) or LDL (**B**). Multiple linear regression models indicated lipid adjusted TCDD levels negatively correlated with cholesterol and LDL.

Future Goals

- Transfect modified AHR into Hepa cells
- Use qRTPCR to analyze HMGCR expression after treating wild type AHR, AHR -/-, R554L, and P554L/P275K with TCDD

References

[1] Denison, M.S., et al., *Chemico-Biological Interactions*, **2017**, *141*(1-2), 3-24
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Acknowledgments

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