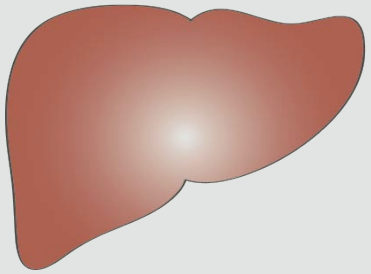
MICHIGAN STATE UNIVERSITY

AhR regulation of Scd1 and alterations in hepatic lipid metabolism



Michelle Angrish
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Genetics and Integrative Toxicology Ph.D. Programs

Outline

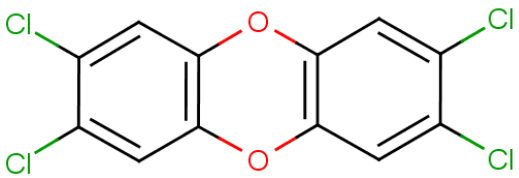
- Dioxin: TCDD
- Aryl hydrocarbon receptor: AhR
- Stearoyl-CoA desaturase 1: Scd1
- AhR regulation of Scd1
- Alterations in hepatic lipid metabolism
- Significance

Dioxin

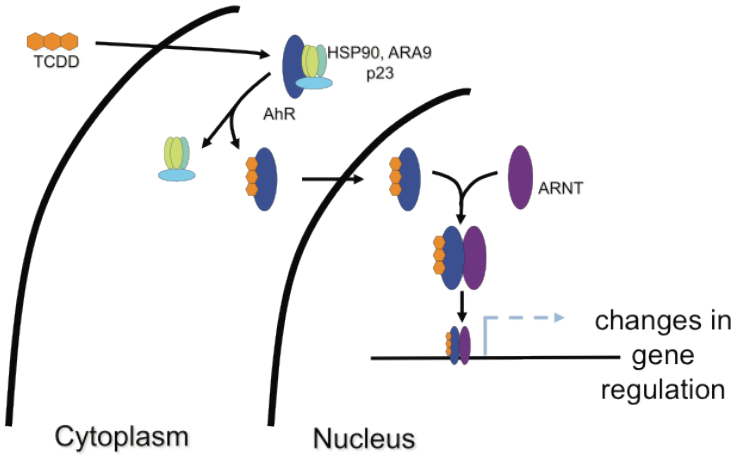
- Ubiquitous environmental contaminant
  - Primary human exposure through consumption of animal products (meat, fish, & dairy)
- Sources
  - Industrial
    - processes involving chlorine, i.e. pulp and paper bleaching
  - Natural
    - volcanoes, forest fires
  - Other
    - backyard burning of petroleum-based products

Dioxins: TCDD is the most toxic

- 2,3,7,8-Tetrachlorodibenzo-ρ-dioxin (TCDD)
  - **T**oxic **E**quivalency **F**actor (TEF) = 1
  - half-life = 8 yrs in humans



- AhR: mediates most of TCDD's biological effects
  - AhR, aryl hydrocarbon receptor
  - ARNT, AhR nuclear translocator

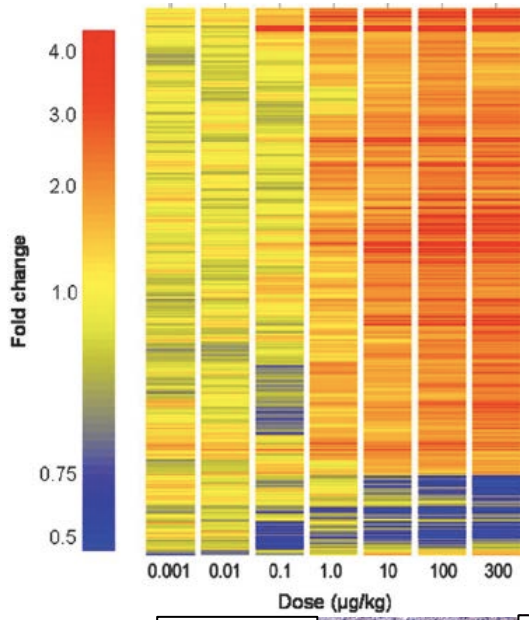


TCDD biological effects

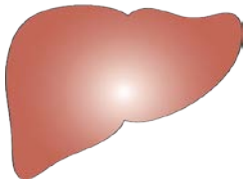
- Hepatic effects
  - hepatocellular carcinoma
  - fatty infiltration (steatosis)

- Effects in humans
  - steatosis
  - chloracne

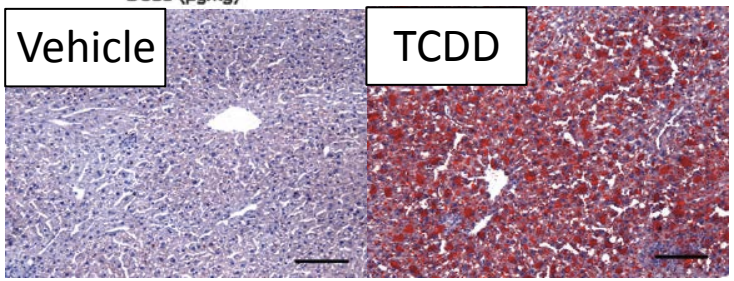
- Hepatic effects in mice



◀Left: altered gene expression



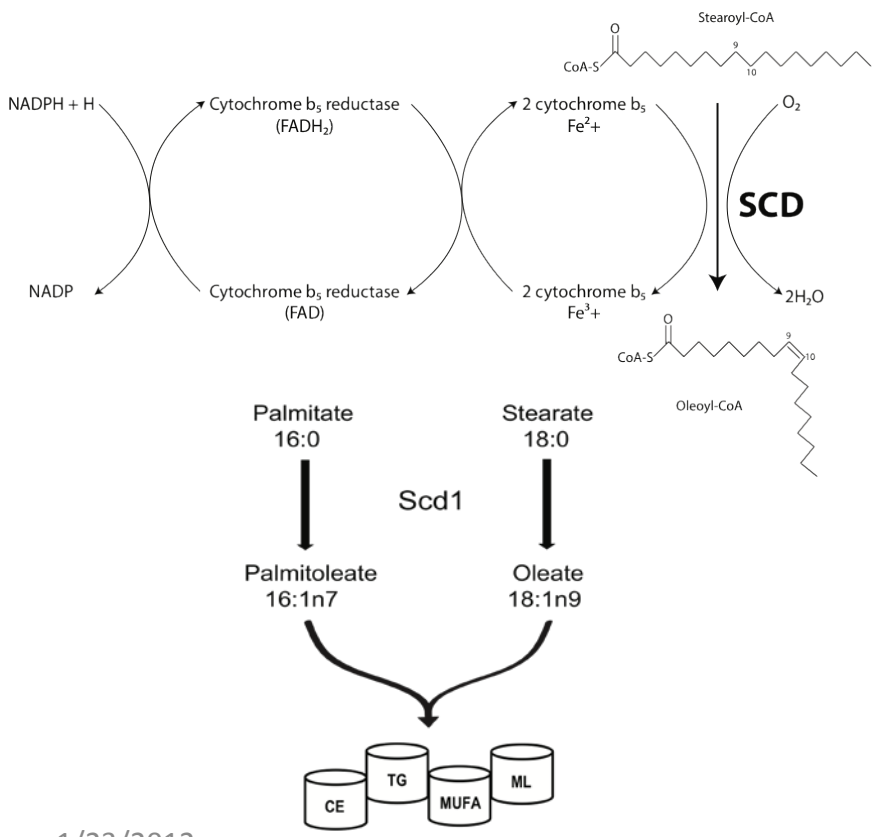
▶Right: steatosis



Boverhof *et al.*, 2005, 2006

Stearoyl-CoA desaturase 1 (Scd1)

- Scd1 catalyzes the rate-limiting step in MUFA formation



- Mice
  - Scd1 null mice are resistant to diet-induced steatosis
- Humans
  - SCD activity is associated with hypertriglyceridemia, insulin resistance, and obesity
  - Polymorphisms in SCD are associated with body fat distribution and insulin sensitivity
  - SCD expression is implicated in human cancer

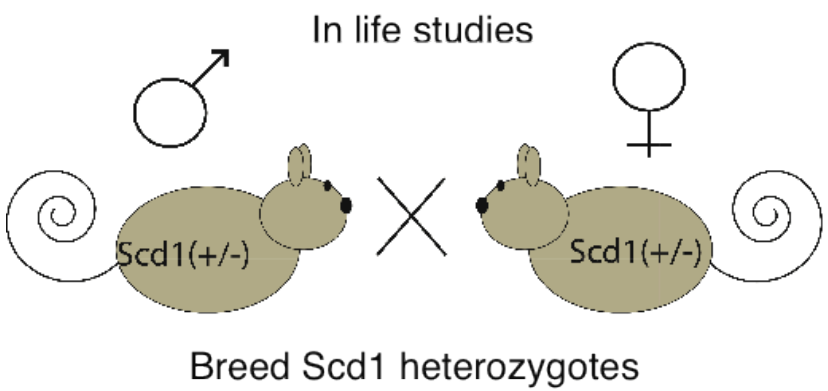
Hypothesis and research questions

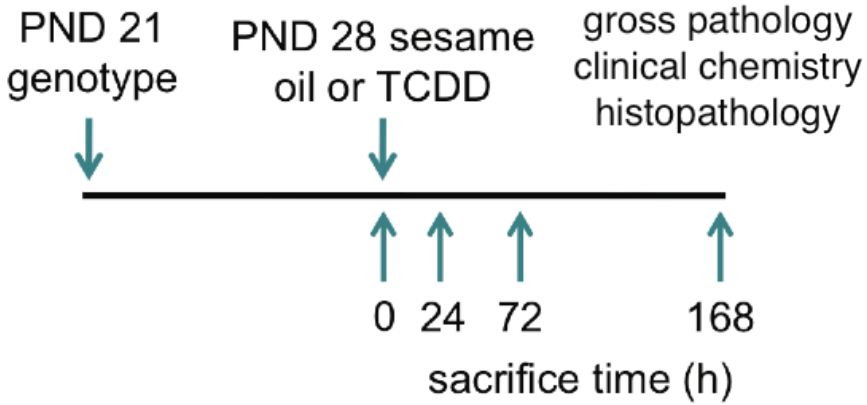
- Scd1 deficiency protects from TCDD-induced steatosis

1. Examine AhR regulation of Scd1

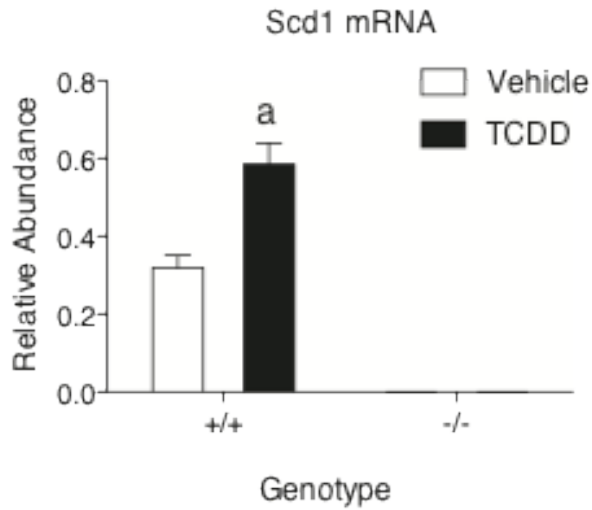
2. Characterize TCDD-induced steatosis in Scd1 wild-type and null mice

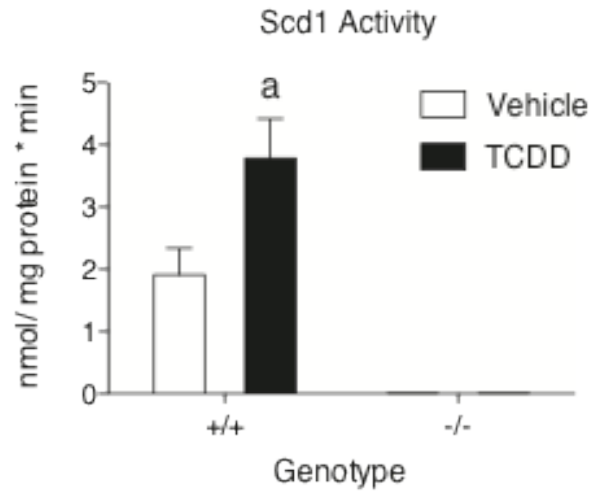
Approach

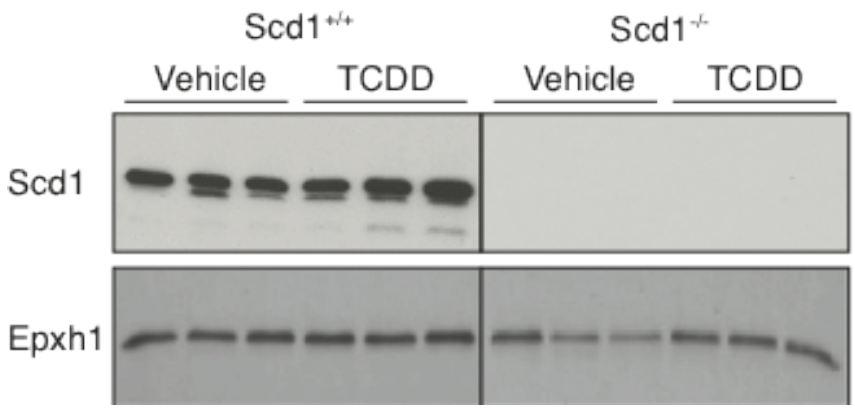

In life studies
Scd1(+/-)
Scd1(+/-)
Breed Scd1 heterozygotes

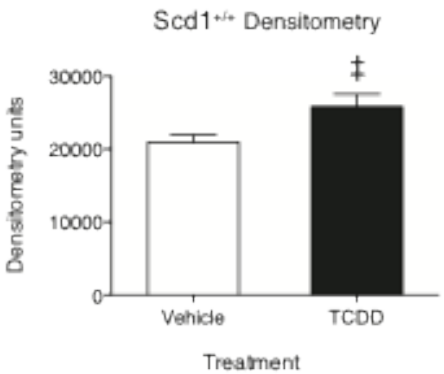

PND 21
genotype
PND 28 sesame
oil or TCDD
gross pathology
clinical chemistry
histopathology
0
24
72
168
sacrifice time (h)

TCDD induces Scd1 mRNA, protein, and activity levels



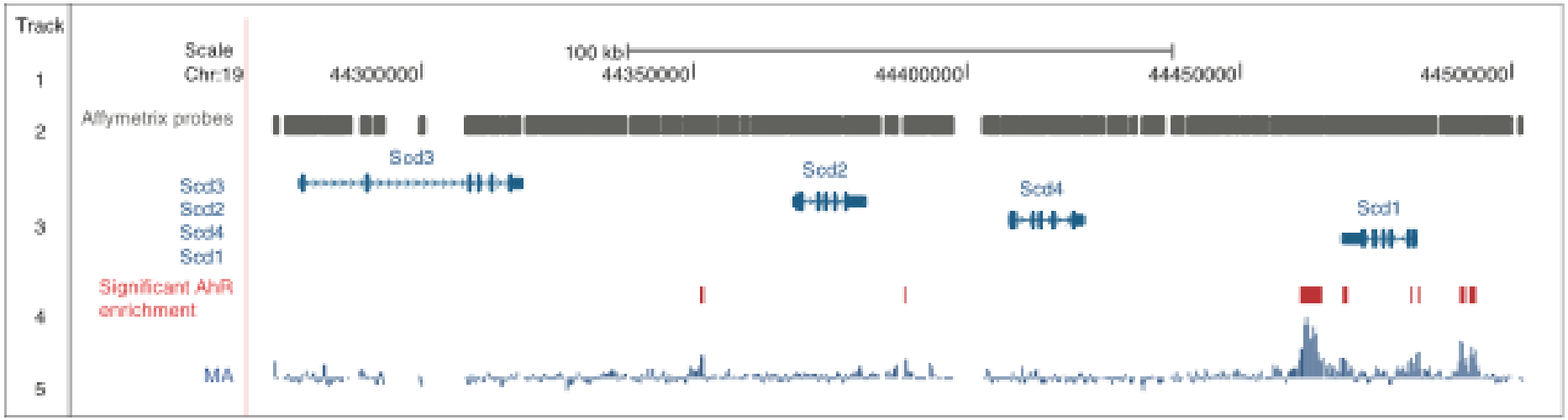






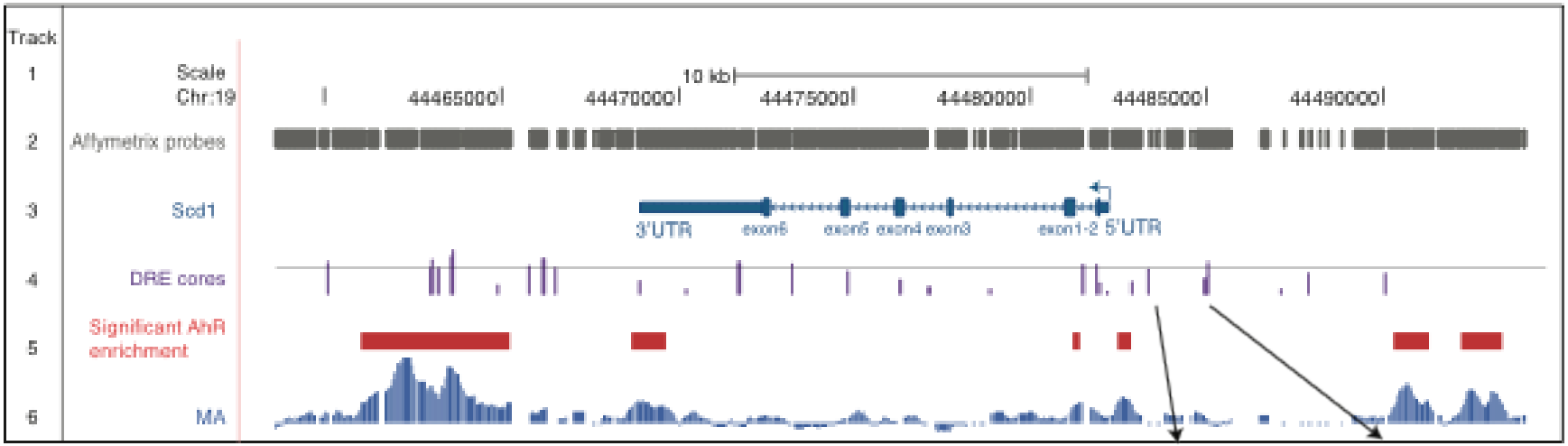
a for p = 0.05 TCDD vs. Vehicle, ‡ for p = 0.08 vs. Vehicle

TCDD-induced AhR recruitment at *Scd1-4* loci



Track 1: scale and chromosome position
Track 2: probe locations
Track 3: gene organization
Track 4: regions of significant (FDR<0.01) AhR enrichment
Track 5: histograms depicting moving average (MA)

AhR enrichment and DRE distributions at *Scd1* loci



Above:

Track 1: scale and chromosome position

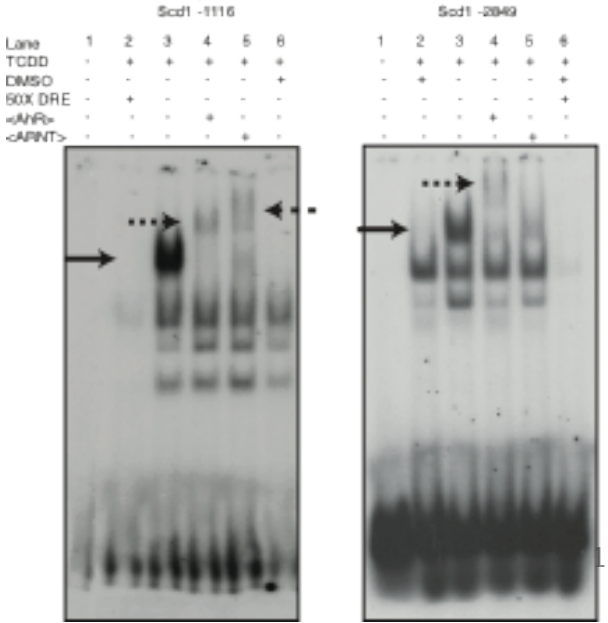
Track 2: probe locations

Track 3: gene organization

Track 4: DRE (5'-GCGTG-3') locations. Height of bar = matrix similarity score (MSS), horizontal line indicates MSS of 0.8

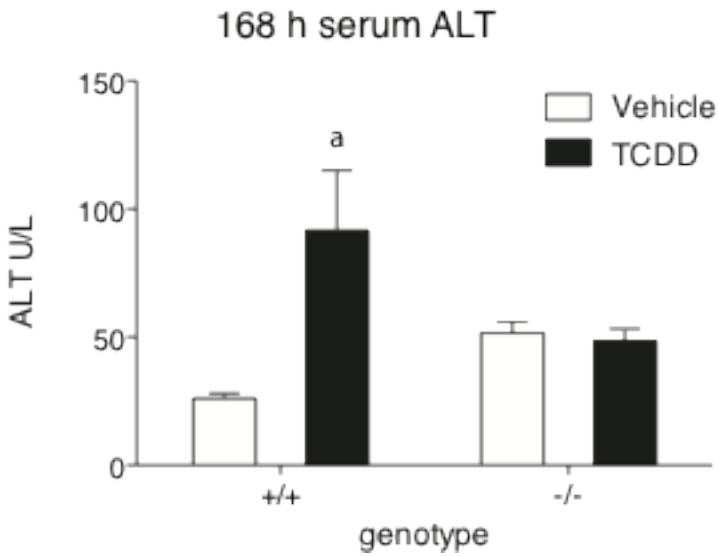
Track 5: regions of significant (FDR<0.01) AhR enrichment

Track 6: histograms depicting moving average (MA)



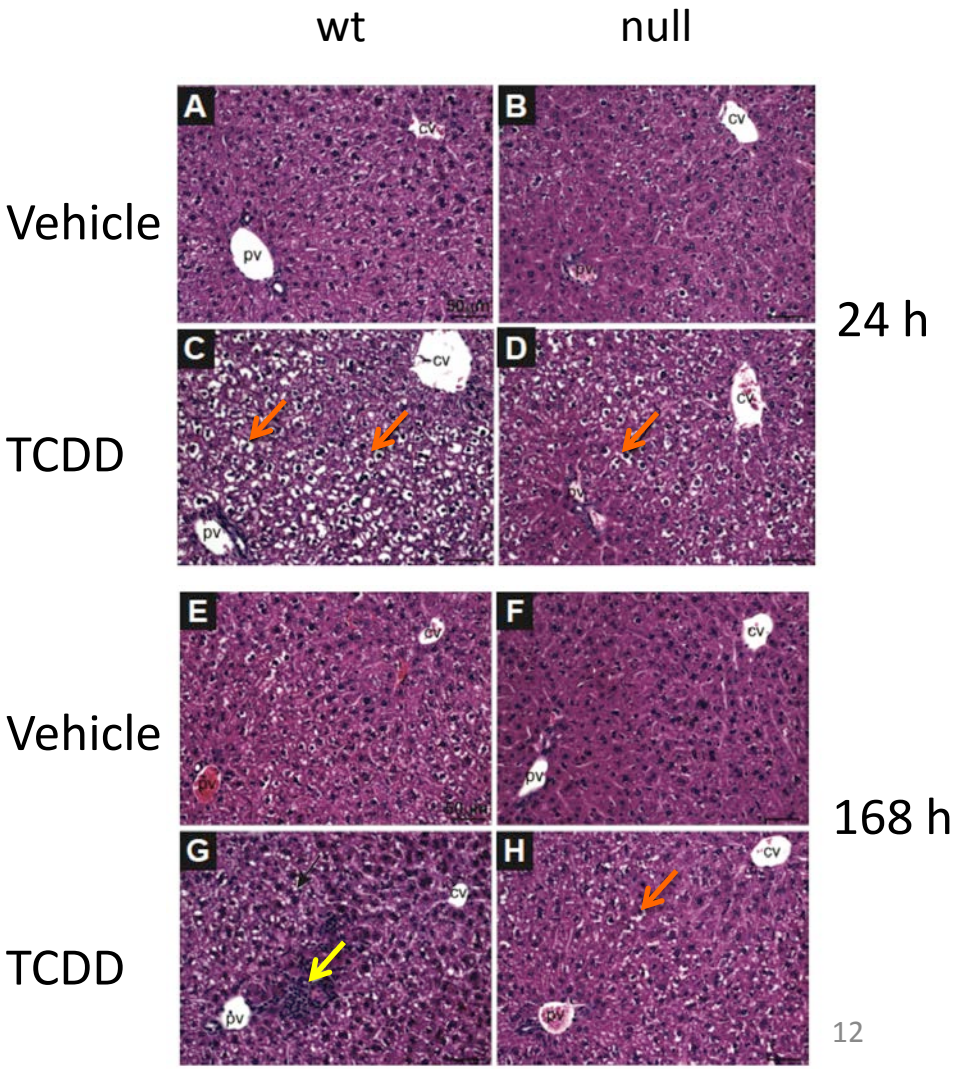
Right: Band shift assays

Scd1 exacerbates TCDD-induced hepatotoxicity at 168 h post-dose



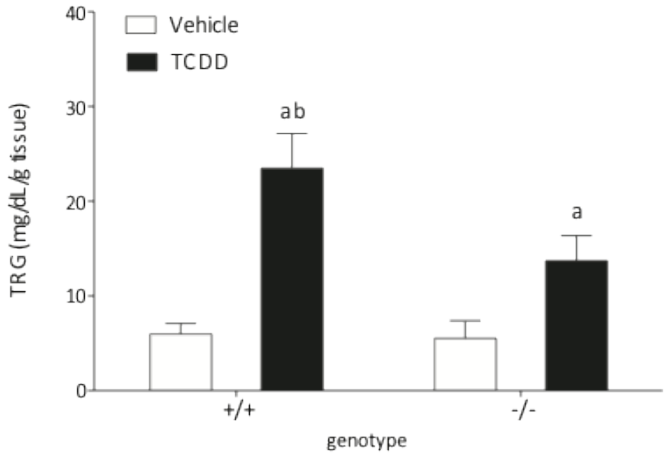
TCDD induces ALT in wt mice. Scd1 deficiency attenuates vacuolization (orange arrow) and inflammation (yellow arrow).

a $p < 0.05$ TCDD vs. Vehicle within a genotype
b $p < 0.05$ Scd1+/+ vs. Scd1-/- TCDD

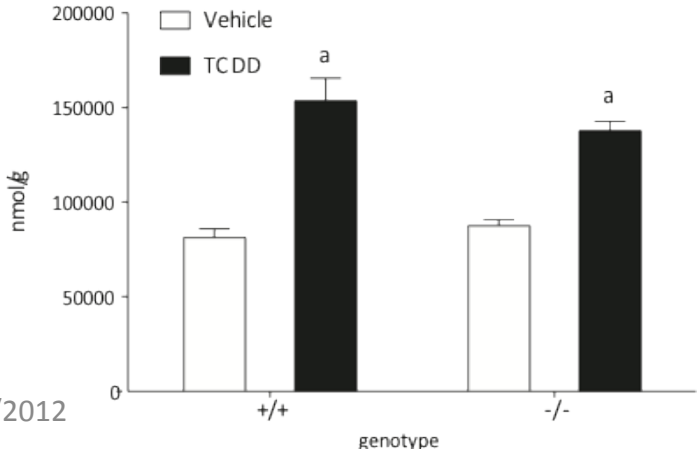


Lipid levels and composition 168 h post-dose in $Scd1^{+/+}$ and $Scd1^{-/-}$ mice

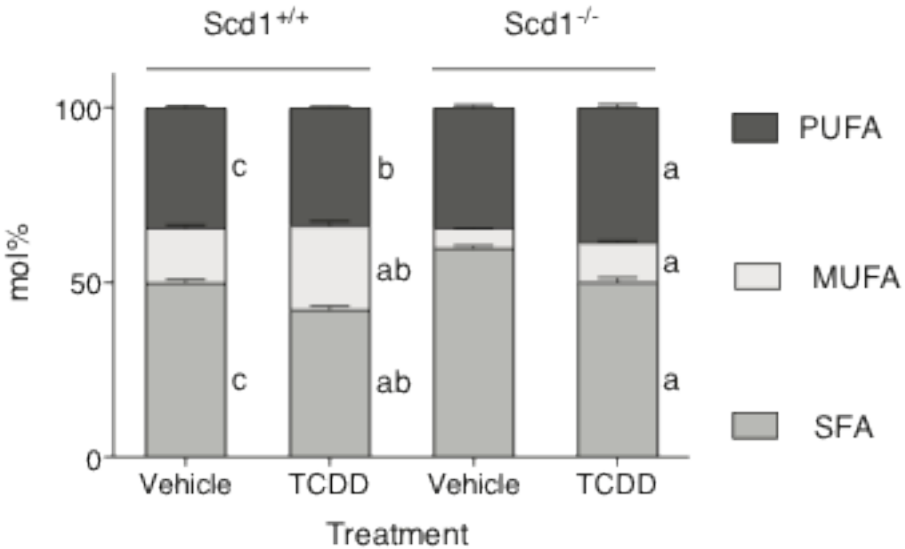
Triglyceride Levels



Total Fatty Acid Levels



Fatty Acid Composition



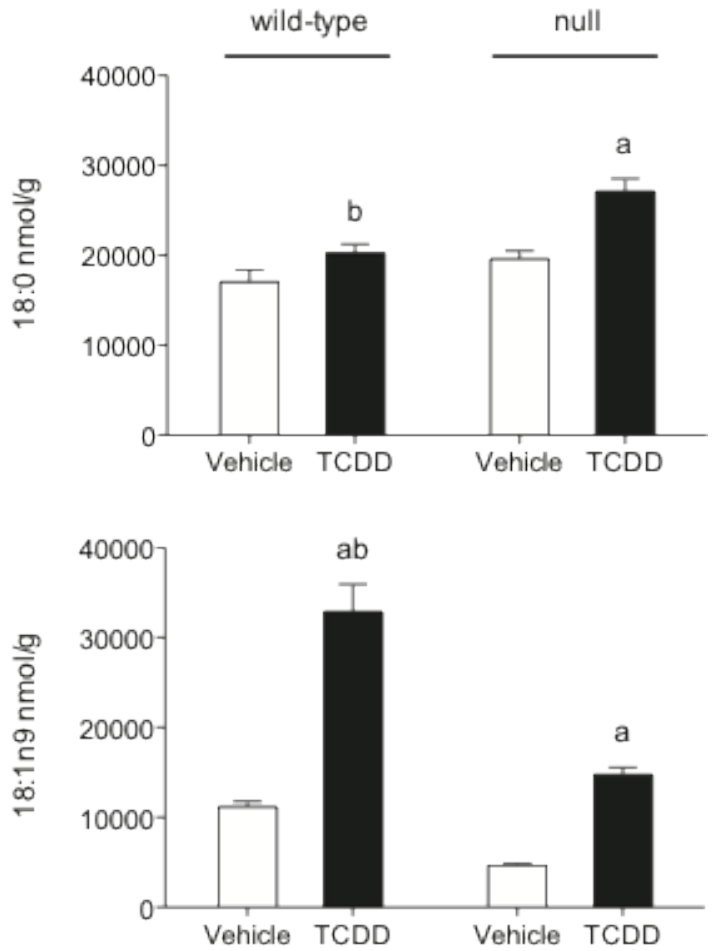
a $p < 0.05$ TCDD vs. Vehicle within a genotype
b $p < 0.05$ Scd1+/+ vs. Scd1-/- TCDD
c $p < 0.05$ Scd1+/+ vs. Scd1-/- Vehicle

18:0 and 18:1 hepatic levels

18:0

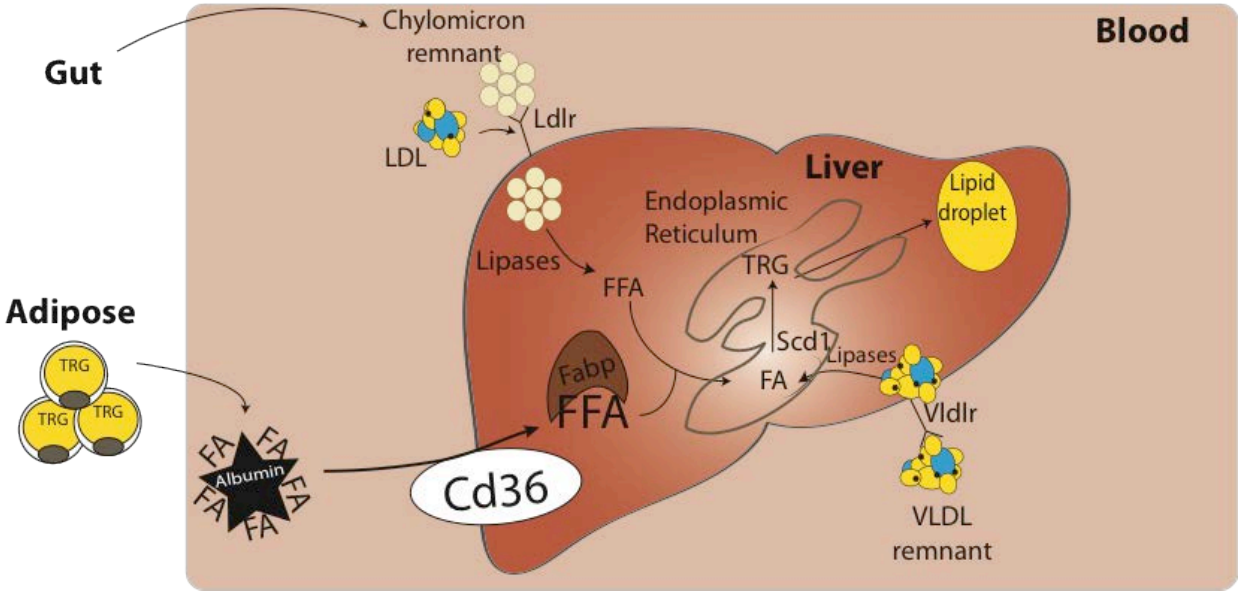
↓ **Scd1**

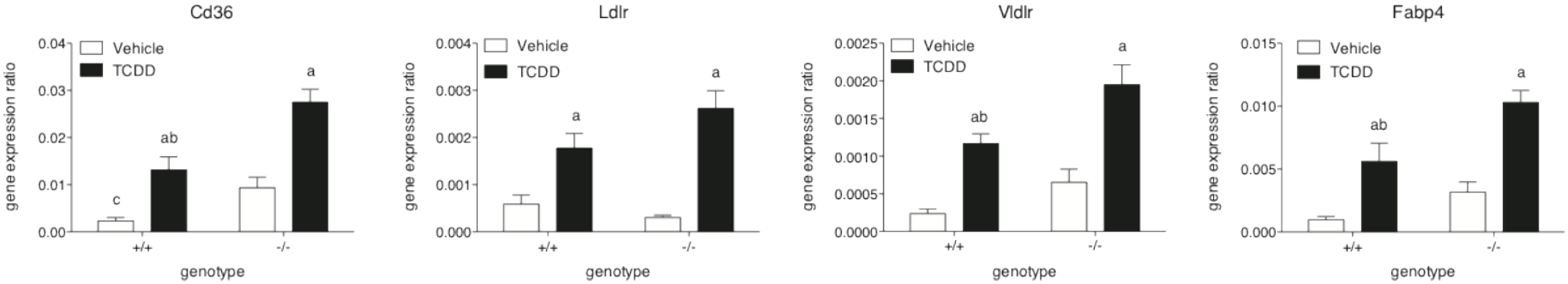
18:1n9



a $p < 0.05$ TCDD vs. Vehicle within a genotype
b $p < 0.05$ Scd1+/+ vs. Scd1-/- TCDD

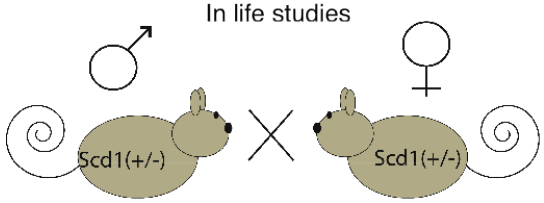
TCDD's effects on hepatic lipid transport in Scd1$^{+/+}$ and Scd1$^{-/-}$ mice



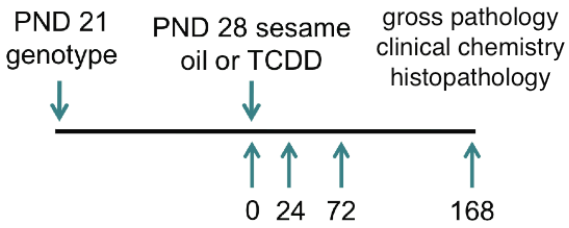


a $p < 0.05$ TCDD vs. Vehicle within a genotype
b $p < 0.05$ Scd1+/+ vs. Scd1-/- TCDD
data reported 168 h post-dose

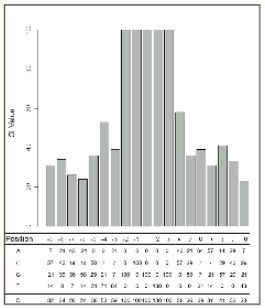
Summary



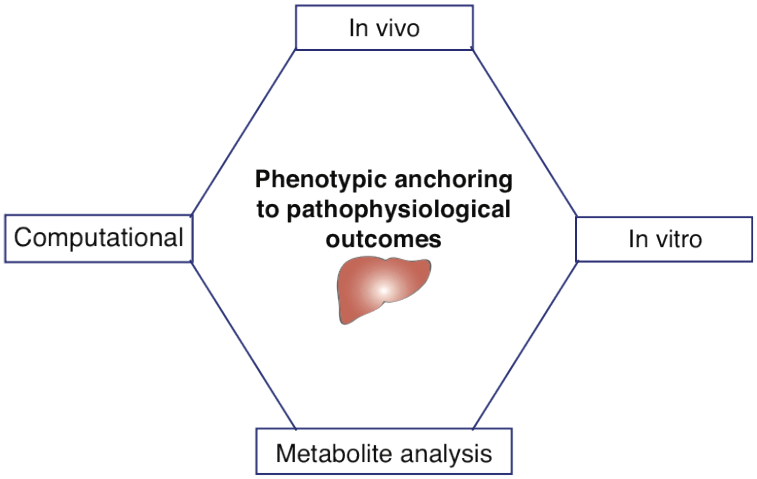
Breed Scd1 heterozygotes



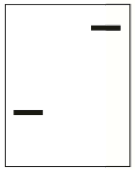
Position Weight Matrix



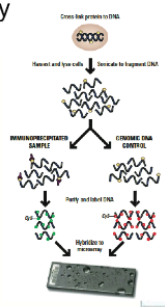
DRE locations
& distrbutions



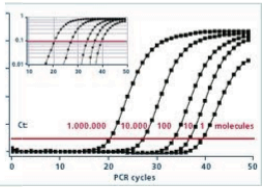
Band Shift Assay



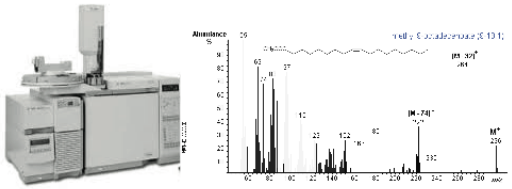
ChIP-chip



QPCR



DRE/AhR binding
Genome-wide AhR enrichment
Gene expression
Activity Assays

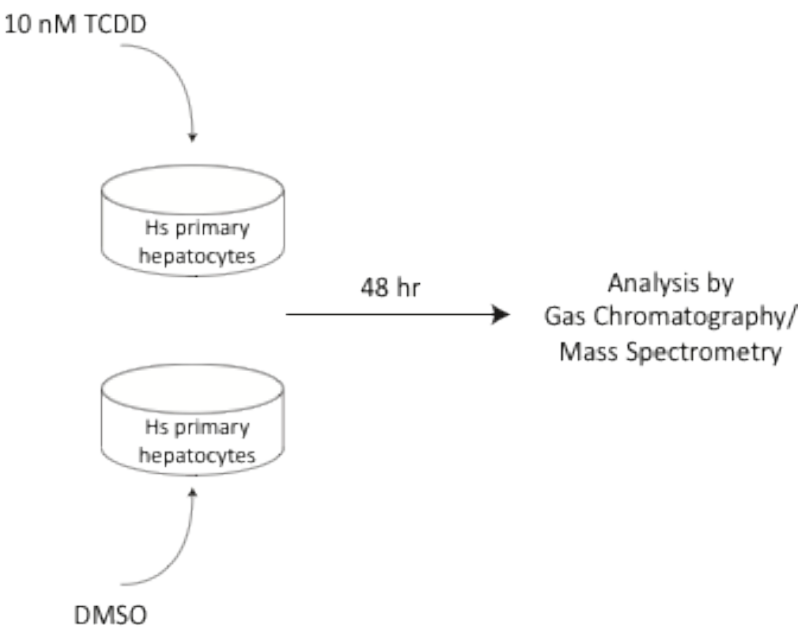


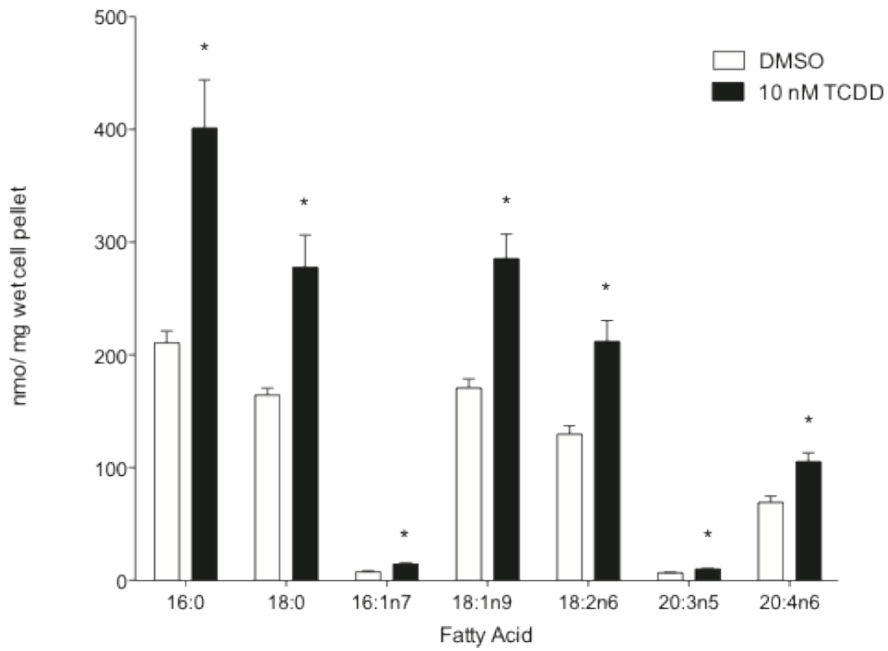
GC-MS of hepatic lipids

Human relevance

- Primary cell culture

  Are lipid profiles in human hepatocytes altered by TCDD exposure?

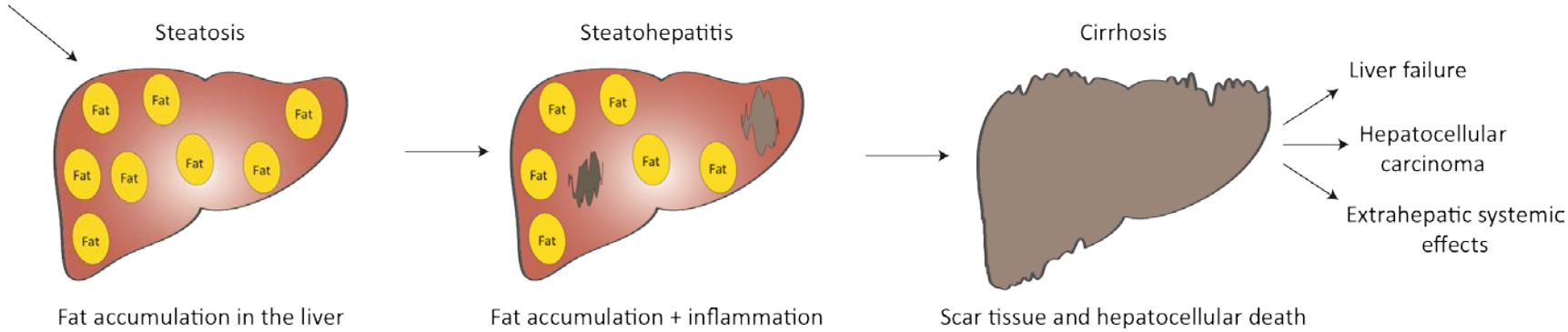




* $p < 0.05$ TCDD vs. DMSO

Human health implications

AhR ac :ion



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Dr. Lyle Burgoon

ChIP-chip
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University of Toronto

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