# Using *In Silico* Methods to Examine Atherosclerosis in the ApoE KO Mouse: a Basic Science Meta-Analysis

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## Introduction

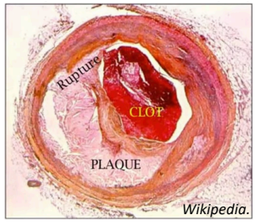
- Atherosclerosis is a chronic blood vessel disease that causes heart attack and stroke,
- the leading causes of death worldwide.
- The apolipoprotein E (ApoE) knockout mouse is the most widely used preclinical model of atherosclerosis.
- Since 1995, over 6,000 studies have been published using the ApoE KO model system.

## Hypothesis

A systematic analysis of genes that have been experimentally implicated in atherosclerosis may identify common regulators and pathways of atherosclerosis in the ApoE KO mouse.

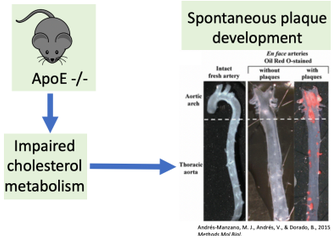
## Background: Atherosclerosis and the ApoE KO Mouse

**Cross-section of a human coronary artery with atherosclerotic plaque:**




- Large lipid core, high inflammatory cell content.
- Inflamed plaques with greater lipid content are more likely to rupture, leading to heart attack, stroke.

**Plaque development in the ApoE KO mouse:**




- Apolipoprotein E (ApoE) is involved in lipid transport and metabolism.
- Mice deficient for ApoE develop plaques with similar composition to those seen in humans (lipid core, macrophage rich).

## Methods

1. PubMed search for all ApoE KO studies published in *Atherosclerosis, Thrombosis, and Vascular Biology* (ATVB) from 1995 to 2019 (~10% of all published ApoE studies).
2. Entry of methods and results of each paper into RedCap collection database.
3. Selective inclusion of records from ApoE KO mouse atherosclerosis studies with single gene perturbation.
4. Transformation of dataset for Ingenuity Pathway Analysis (IPA) upload.
5. Systematic analysis of dataset using IPA's Core Analysis function.

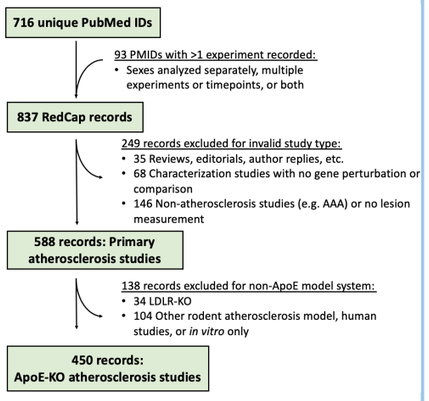
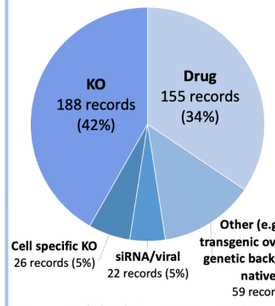



*Figure 1: Step 3, exclusion flowchart.*

## Results: Plaque Measurement and Intervention Demographics

**A. Intervention type in ApoE KO mouse atherosclerosis records**




**90 records excluded from IPA analysis for invalid gene perturbation:**

- 69 Unknown gene target (e.g. dietary supplement study)
- 12 Multi-target drug or multi-gene knockout
- 3 Non-native gene (e.g. fusion protein)
- 6 No human NCBI gene symbol available

**B. Plaque measurements taken in records included in IPA analysis**

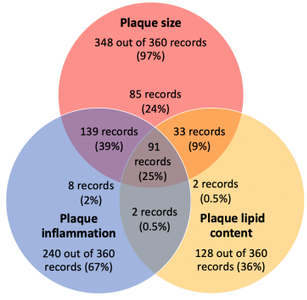


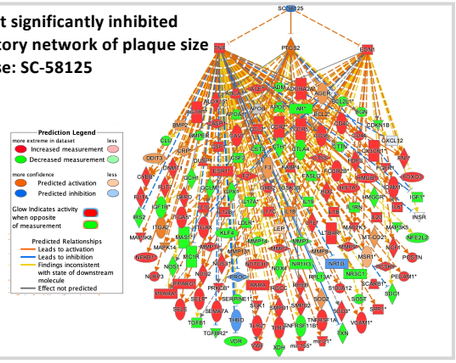

*Figure 2: (A) Of the 450 ApoE KO mouse atherosclerosis records, the greatest proportion of interventions were knockout and drug studies. (B) Nearly all records included in our IPA analysis came from studies that measured plaque size, about two-thirds measured inflammation, and about one-third measured lipid content.*

## Results: Upstream Regulators and Enriched Pathways of Plaque Size and Composition

**A. Top 3 master regulators of plaque size**

| Master Regulator | Molecule Type | Predicted Activation | Activation z-score | p-value of overlap |
|---|---|---|---|---|
| SC-58125 | chemical drug | Inhibited | -2.835 | 4.55E-85 |
| sesamol | chemical - endogenous mammalian | Inhibited | -2.835 | 2.42E-82 |
| TNF | cytokine | Activated | 2.525 | 6.05E-80 |

**B. Most significantly inhibited regulatory network of plaque size increase: SC-58125**




**C. Top 5 pathways of plaque size and composition**

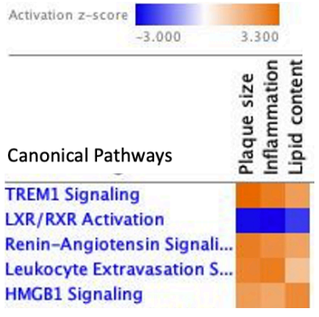
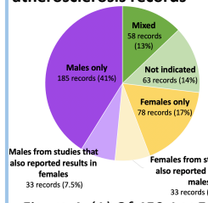


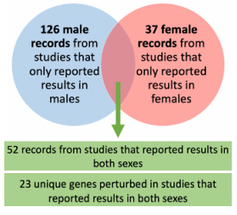

*Figure 3: (A) The three most significant upstream regulators of plaque size were SC-58125, sesamol, and TNF. SC-58125, sesamol, and TNF regulate COX-2, implicating COX-2 as a potential master regulator of plaque size. COX-2 promotes synthesis of prostacyclin, which has vasodilative and anti-thrombotic effects. Prostacyclin also inhibits leukocyte activation and adhesion, platelet aggregation, and vascular smooth muscle cell proliferation. (B) The SC-58125 regulatory network was significantly inhibited. This network includes the most inhibited (SC-58125) and most activated (TNF) upstream regulators. 134 unique downstream genes, over one-third of genes uploaded to IPA, are represented across the top three regulatory networks. (C) TREM1 and LXR/RXR signaling pathways were significantly enriched. TREM1 signaling, which stimulates inflammatory responses of cytokines and chemokines, was predicted to be activated with gene changes associated with increased plaque size and composition. LXR/RXR signaling was inhibited. LXR/RXR signaling induces the expression of genes involved in lipid metabolism and modulates inflammatory responses in macrophages.*

## Results: Sex Differences

**A. Animal sex in ApoE KO mouse atherosclerosis records**




**B. Studies reporting results in both sexes**




52 records from studies that reported results in both sexes

23 unique genes perturbed in studies that reported results in both sexes

**C. Top 5 pathways of plaque size and composition by sex, sorted by trend**

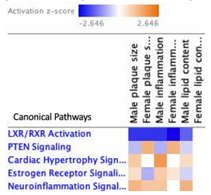
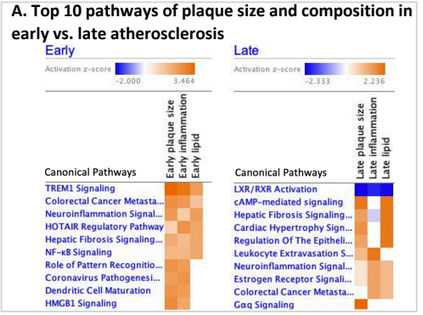



*Figure 4: (A) Of 450 ApoE KO mouse atherosclerosis records, the greatest proportion of records came from studies that only looked at male mice. Only 25% of records reported results in female mice. (B) Of 360 records included in our IPA analysis, 52 records reported results of a single gene perturbation in both male and female mice. (C) PTEN and Estrogen Receptor Signaling were oppositely regulated by sex. In female mice, inhibition of ESR signaling was associated with increased plaque size and composition, suggesting that ESR signaling may have an atheroprotective effect in females. PTEN signaling was inhibited in males but activated in females, which suggests a novel role for PTEN signaling as a mechanism that contributes to sex differences in atherosclerosis.*

## Results: Early vs. Late Atherosclerosis

**A. Top 10 pathways of plaque size and composition in early vs. late atherosclerosis**




**B. Top 10 diseases and functions of plaque size and composition in early vs. late atherosclerosis**

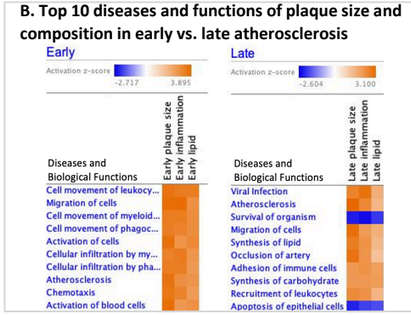



*Figure 5: (A) Inflammatory pathways are activated in early atherosclerosis. LXR/RXR signaling is inhibited in late atherosclerosis. (B) Diseases and biological functions associated with cell movement are activated early. Functions associated with survival and apoptosis signaling are inhibited late.*

## Conclusions

Summary:

- By integrating results from ~10% of ApoE KO mouse atherosclerosis studies, common upstream regulators and pathways, differences in males vs. females and early vs. late atherosclerosis were identified.
- COX-2 is a potential master regulator of gene changes associated with plaque size and composition. This finding may be controversial, as COX-2 inhibitors are associated with increased cardiovascular risk. Further investigation is needed to understand the regulatory role of COX-2 in plaque development.
- Sex differences in atherosclerosis may be explained by differential activation of PTEN and ESR signaling pathways.
- Early atherosclerosis is characterized by the activation of inflammatory pathways and functions. In late atherosclerosis, lipid metabolism and functions associated with cell survival are significantly inhibited.

Future directions:

- Repeat analysis on LDLR KO mouse studies published in ATVB – are the two models equivalent?
- Consider pathways and regulators identified by IPA in the context of human genome-wide sequencing data.

## Limitations

- Dataset contains only ~10% of all published ApoE atherosclerosis studies.
- IPA knowledge database is curated from studies used in our analysis.
- Unable to indicate cell specificity in IPA (i.e. cell specific knockout treated as global knockout).