A GxE QTL on Chr. 15 underlies susceptibility to air pollution-induced lung injury in mice

Adelaide Tovar,[1,2,*] Greg Smith,[1,3] Joe Thomas,[1] Katie McFadden,[1] Jim Wagner,[4] Jack Harkema,[4] and Samir Kelada[1,2,3]

[1]Department of Genetics, [2]Curriculum in Genetics & Molecular Biology, [3]Curriculum in Toxicology & Environmental Medicine, The University of North Carolina at Chapel Hill, Chapel Hill, NC 27599. [4]Department of Pathology & Diagnostic Investigation and Institute for Integrated Toxicology, Michigan State University, East Lansing, MI 48824.

*edialeda@email.unc.edu @ade__lady

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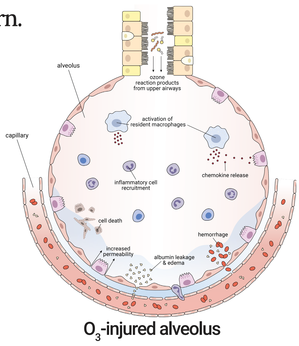
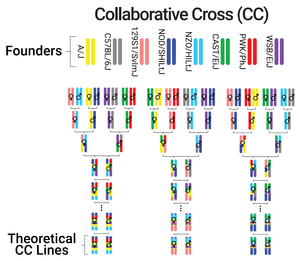
Ambient ozone (O_3) pollution is a critical public health concern.

- Over 40% of the US population resides in areas where the National Ambient Air Quality Standard (NAAQS) for O_3 is regularly exceeded
- O_3 exposure causes airway inflammation, lung tissue injury, and can aggravate existing lung conditions (asthma, COPD)
- Epidemiologic studies have linked O_3 exposure to increased risk of heart attacks and respiratory infections
- Inter-individual differences in the response to ozone exposure have been demonstrated in both humans and rodents
- Previous studies have identified genetic regulators of respiratory responses to ozone exposure (including *Tlr4* and *Tnf*); none have identifed loci responsible for systemic inflammatory responses, and these earlier studies didn't utilize the full range of genetic variation within *Mus musculus*

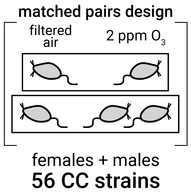


O_3-injured alveolus

We used the Collaborative Cross to identify novel genetic loci that mediate responses to O_3 exposure.



The CC genetic reference panel (left) was generated through funnel inbreeding of 8 founder strains that are representative of 3 *Mus musculus* subspecies.



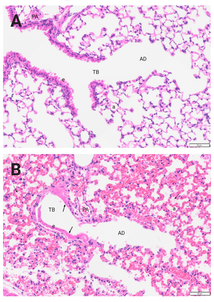
expose 3 hr, necropsy 21 hr later

inflammatory/injury phenotyping
bronchoalveolar lavage
whole blood collection

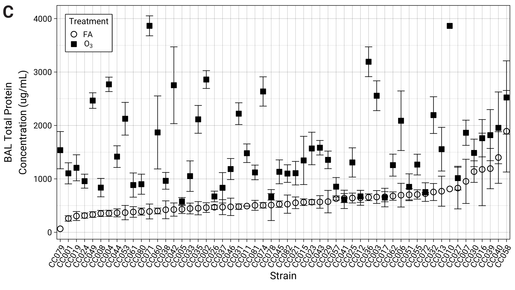
gene expression
tissue harvest

Utilizing a matched pairs design (above), we exposed mice from 56 CC strains to filtered air (FA) or 2 ppm O_3 for 3 hours and collected samples 21 hours later. We used a non-specific marker of lung injury, total protein concentration in bronchoalveolar lavage fluid, to estimate lung permeability.

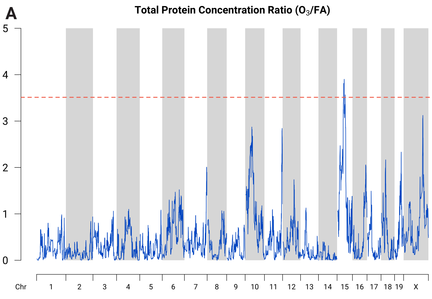
Total protein concentration, a marker of lung injury, is increased after O_3 exposure and highly variable across 56 CC strains.

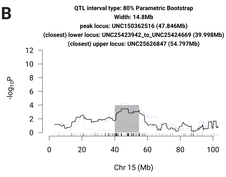


(left) Representative histological sections of the bronchiolar conducting airways of a (A) filtered air and an (B) O_3-exposed mouse from the strain CC071/TauUnc (highest responder), illustrating apparent injury in the O_3-exposed mouse as evidenced by exfoliating necrotic epithelium (solid arrow) and proteinaceous debris (stippled arrow). TB: terminal bronchiole, AD: proximal alveolar duct, PA: pulmonary artery, a: alveolus, e: bronchiolar epithelium. (right) (C) Distribution of total protein concentration. Each point represents a strain mean +/- standard error. (n=4 FA/6 O_3 per strain, on average) (D) Distribution of the log-transformed total protein concentration ratios for all strains. (n=4 matched pairs per strain, on average)

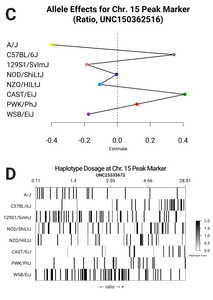


A locus on Chr. 15 is significantly associated with variation in protein (i.e., injury) responses.



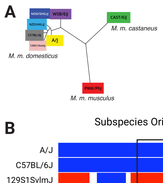


(A, left) Quantitative trait locus (QTL) scan of total protein concentration ratio of the two quantities were generated using regression on probabilities (ROP). Red dashed line indicates the 95% permutation-based significance threshold. Chr. 15 peak locus is depicted in greater detail in (B, above), including the 80% support interval. Proportion of variation explained by the peak locus was 20.96%.

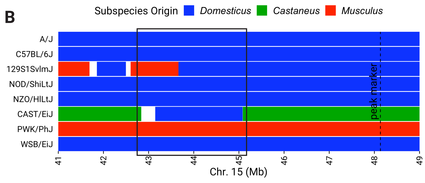


(C) Allele effects estimates at the Chr. 15 peak marker based on regression coefficients. C57BL/6J and CAST/EiJ haplotypes at the locus have a strong positive effect on total protein ratio (in other words, lung injury). (D) Heatmap of the founder haplotype dosages at the peak marker. Each tick mark represents a matched pair where its position along the x-axis corresponds to the phenotype value (ratio) and tick mark shading corresponds to haplotype dosage at the marker.

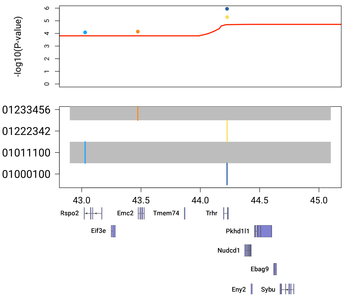
A ~2 Mb region of domesticus introgression in the CAST/EiJ genome contains a candidate gene of interest.

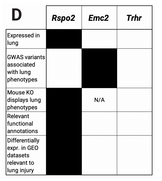


(A, left) Phylogeny of the 8 CC founders, illustrating that C57BL/6J and CAST/EiJ represent two separate *Mus musculus* substrains: *domesticus* and *castaneus*, respectively.



(B, bottom-left) Subspecies origin map of the 8 founder strains in a subregion of the Chr. 15 locus. Coordinates have been lifted over to GRCm38 (mm10). Peak marker is denoted by a dashed line at ~48.1 Mb. A ~2 Mb region of *Mus musculus domesticus* introgression in the CAST/EiJ genome is highlighted and contains 10 protein-coding genes. (C, right) Merge analysis was performed within the ~2 Mb region of *Mus musculus domesticus* introgression to identify candidate causal variants whose strain distribution pattern (SDP) provided a more parsimonious fit than the 8-founder haplotype model used for QTL mapping. Four variants with more significant p-values than the 8 haplotype model were identified: one in *Rspo2*, one in *Emc2*, and two in *Trhr*.





(D, above) A variety of criteria were used in a weight of evidence approach to select *Rspo2* as a candidate gene underlying the Chr. 15 QTL. Only genes with a significant merge variant are displayed and are listed in order of physical position. Black boxes denote affirmative while white boxes denote negative.

Summary

- Using 56 strains of the Collaborative Cross genetic reference population, we identified a locus on Chr. 15 associated with lung injury after acute O_3 exposure
- CC strains with C57BL/6J or CAST/EiJ haplotype at this locus tend to have more total protein in bronchoalveolar lavage (i.e., lung injury) after O_3 exposure than other strains
- *Rspo2* is the lead positional and functional candidate gene of interest within the locus
- Future studies in fibroblasts and knockout mice will be performed to test the plausibility of *Rspo2* as a regulator of responses to acute lung injury

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