National Institute of Environmental Health Sciences
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Identifying G X E Interactions and Thresholds for Toxicity in Diversity Outbred Mice

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Special Volunteer NTP

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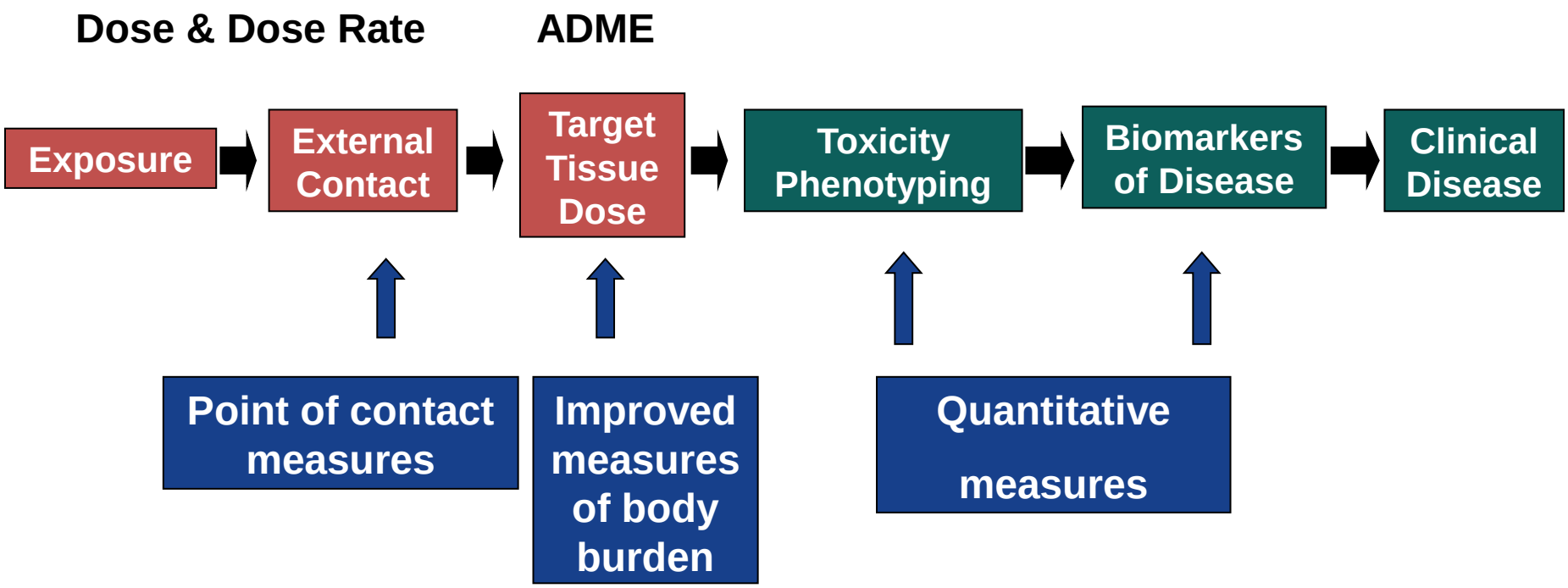
National Toxicology Program
U.S. Department of Health and Human Services

Outline

- **Role and relevance** – inbred homozygous strains vs. Diversity Outbred & CC RIL mice
- **Proof of concept**: Benzene, an environmental genotoxicant and leukemogen
- **Benzene metabolites –-** chromosomal damage associated with the development of leukemia
- **Benchmark Concentration Models** – point of departure
- **Mapping resistance and/or susceptibility QTLs**
- **Future directions**

Genetic and Epigenetic Basis for Susceptibility



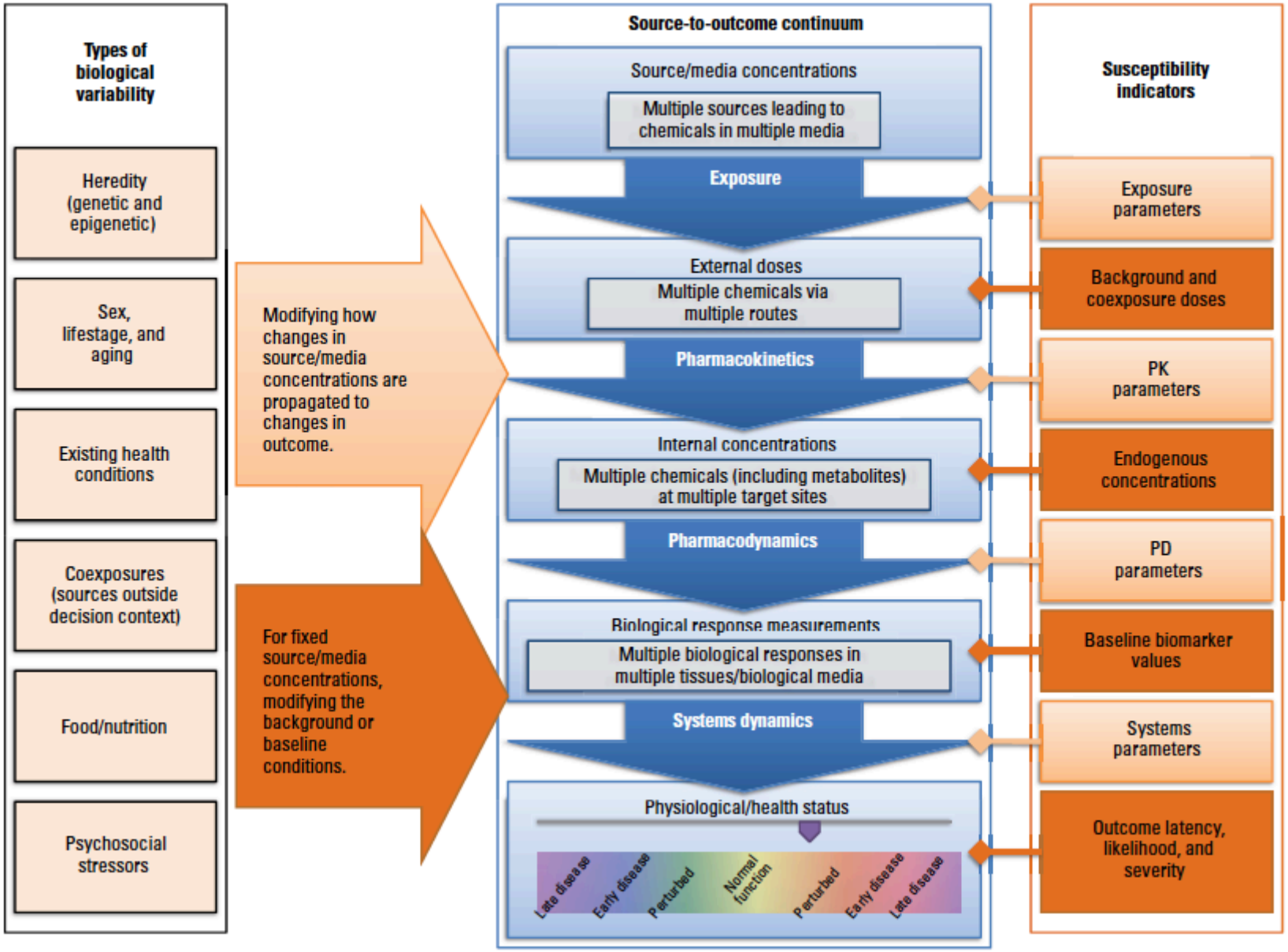
Link individual exposure to biology and to disease

(Adapted from National Research Council, 1987)

Addressing Human Variability in Next-Generation Human Health Risk Assessments of Environmental Chemicals

EHP 121:23 (2013)

Lauren Zeise,[1] Frederic Y. Bois,[2] Weihsueh A. Chiu,[3] Dale Hattis,[4] Ivan Rusyn,[5] and Kathryn Z. Guyton[3]



Brief Background - Toxicogenetics

- Integration of genetics with toxicology
- Inter-individual variation in genetics and environmental exposure (uncontrolled variables)
- Hazard identification and characterization – false negative and false positive error rates?
- Corroborate genetic epidemiology and/or provide an effective tool for safety assessment

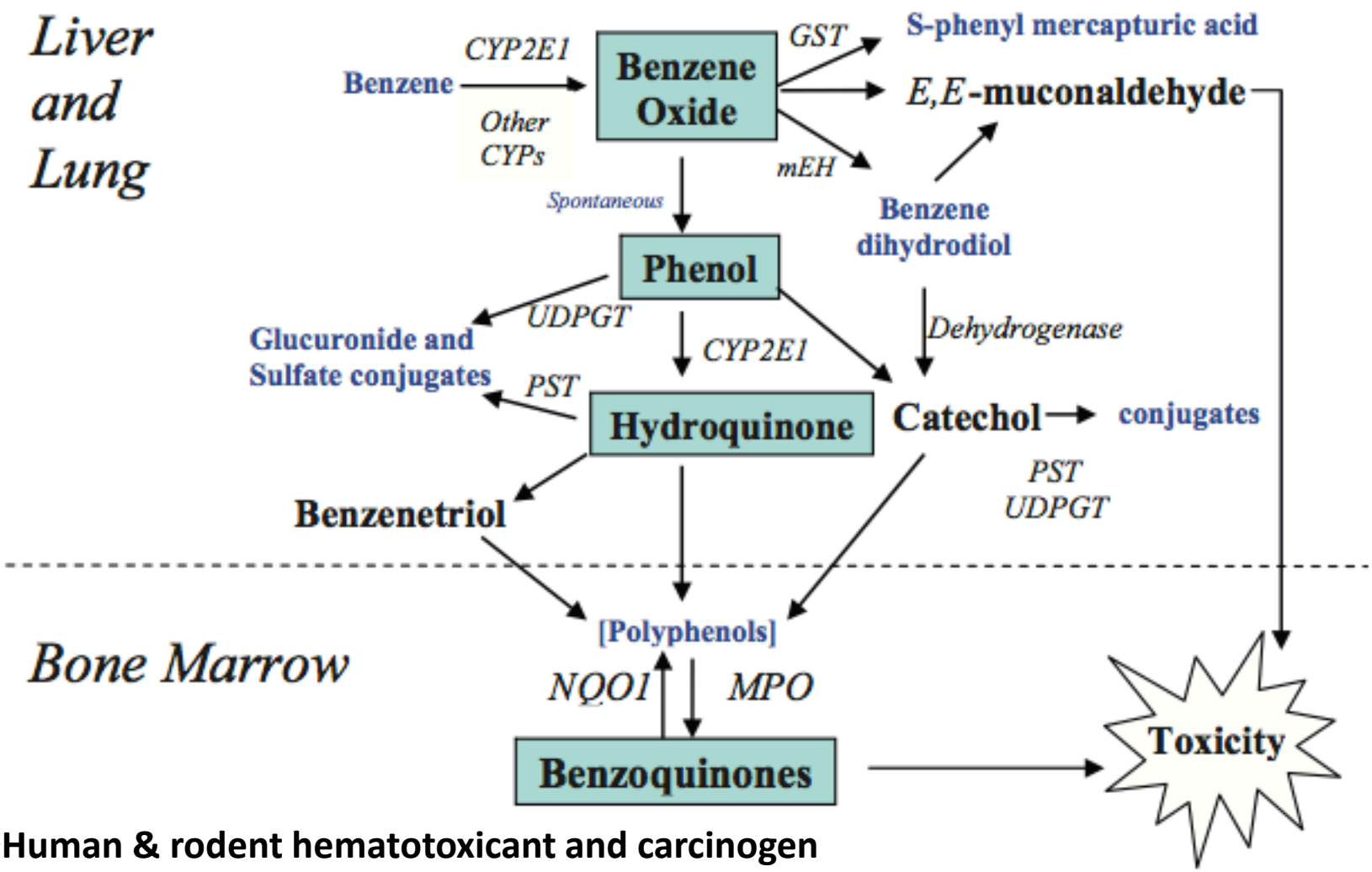
Benzene 28 Day Inhalation Exposure: **Proof of Concept**

- Diversity outbred (J:DO) male mice: 7 & 8th randomly outbred generations; selected from 175 breeding pairs
- Randomly assigned to exposure groups by weight
- Dose levels: 0, 1, 10, 100 ppm benzene, 28 days, 6 hr/day
- 75 male mice per exposure group, 300 mice/study
- 2 independent cohorts to assess reproducibility (600 mice total)
- Endpoints for hematotoxicity and genetic damage
  - % reticulocytes and micronucleated reticulocytes in peripheral blood and bone marrow
  - Mouse Universal Genotyping Array (9K SNPs)
  - Linkage mapping analysis (DO QTLRel)

French et al. EHP (2015) 123:237-245. (Advance online – 6 November 2014)

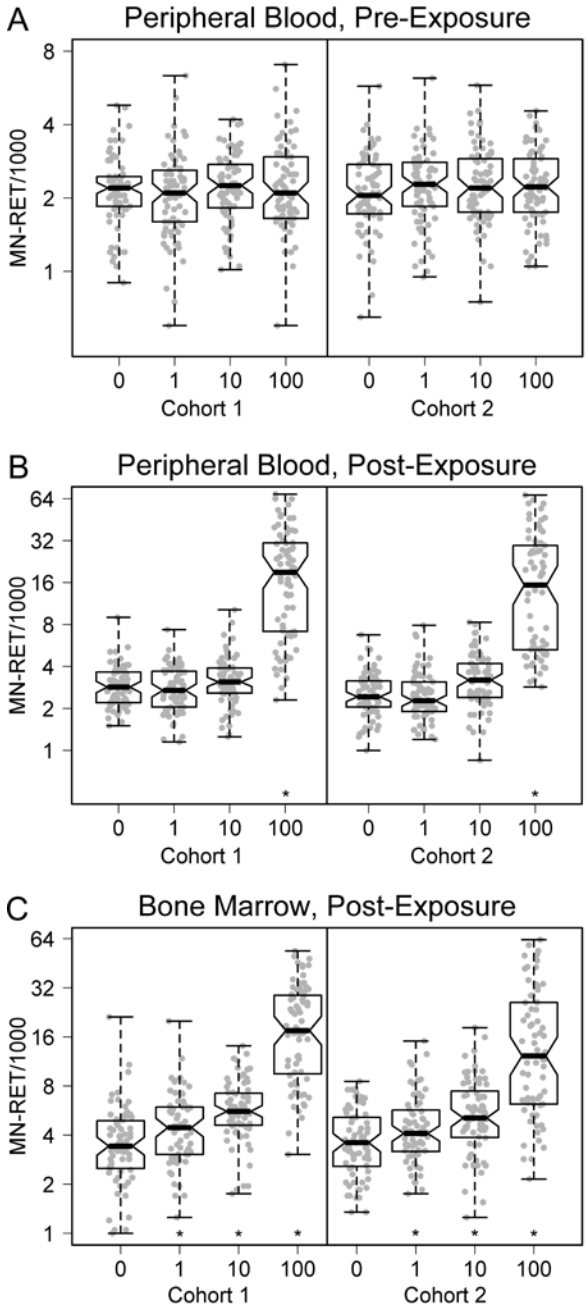
Benzene metabolism and toxicity

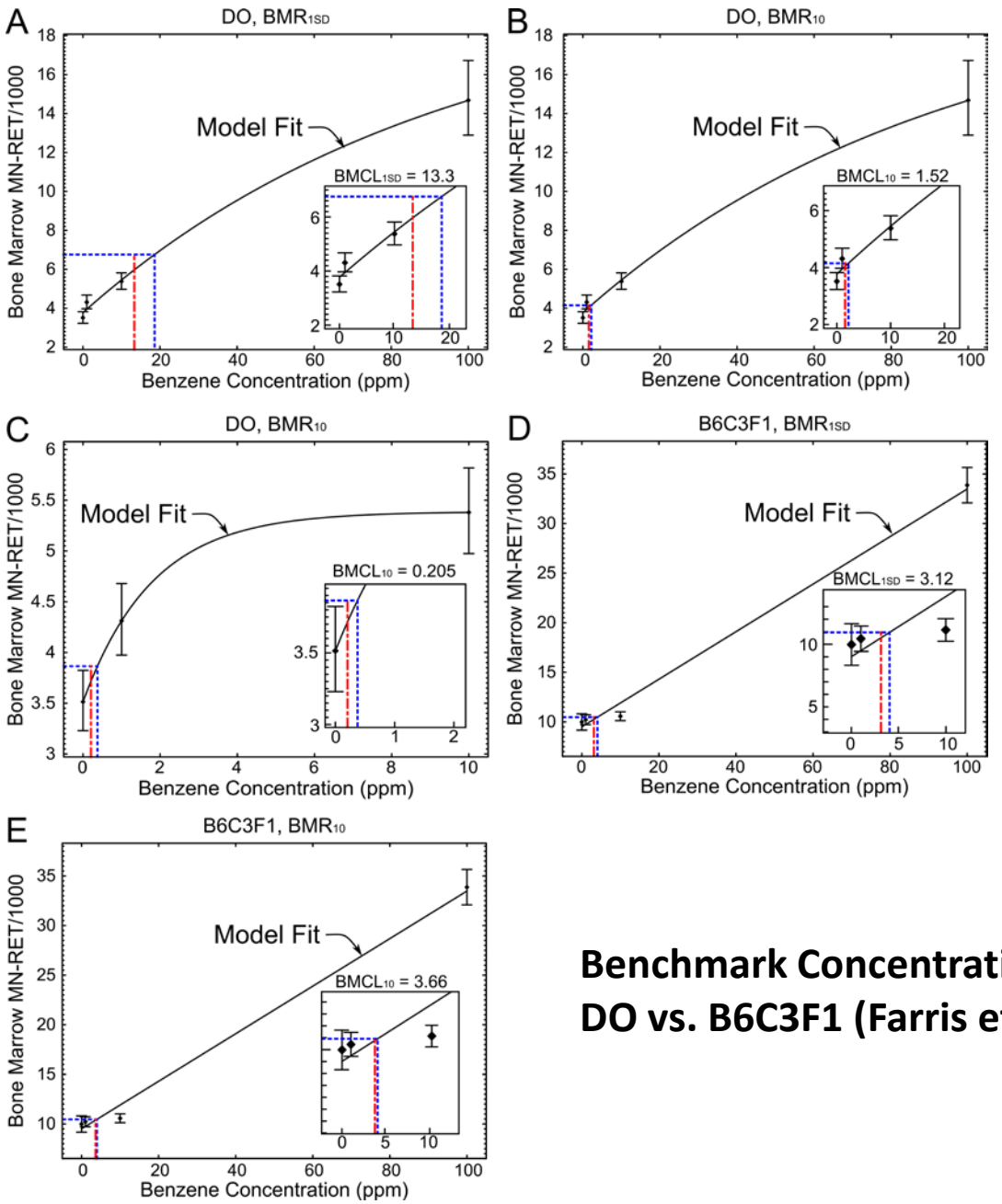
M.T. Smith et al. Chemico-Biological Interactions 192 (2011) 155–159



- **Human & rodent hematotoxicant and carcinogen**
- **Metabolized in liver and bone marrow**
- **Human & rodent ADME (toxicokinetics) are comparable**

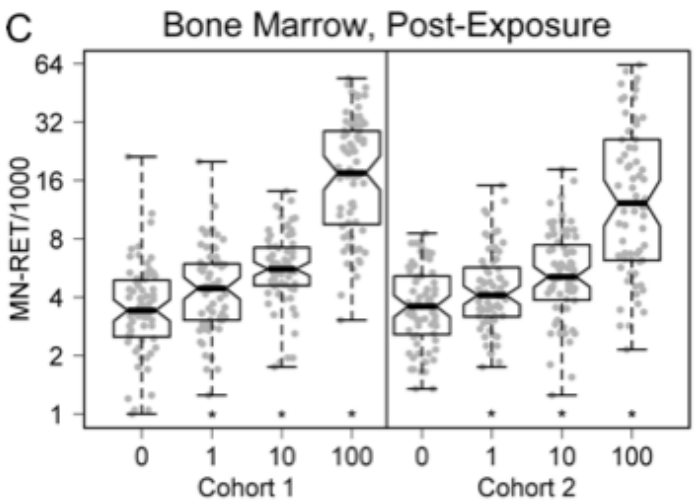
The results….

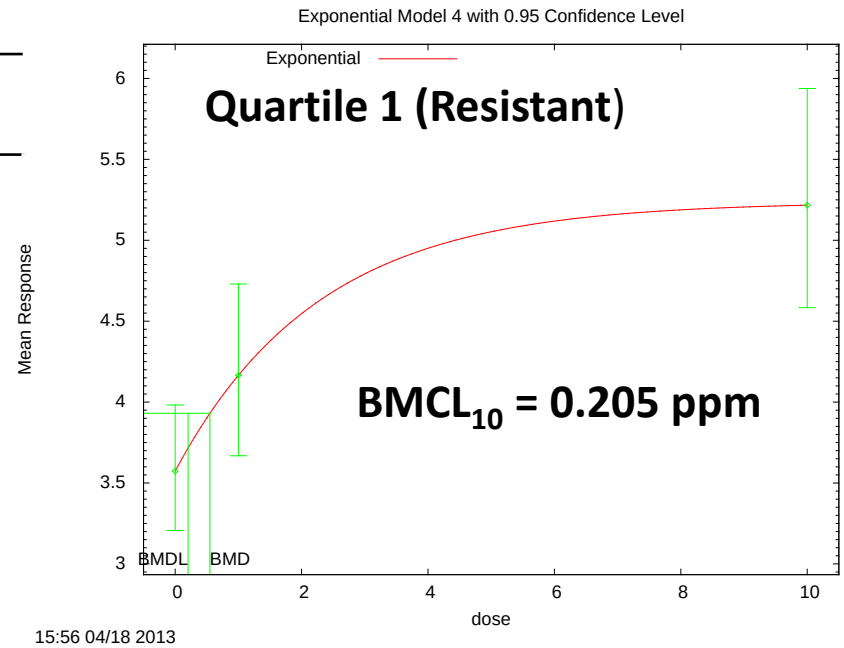

A
Peripheral Blood, Pre-Exposure
MN-RET/1000
8
4
2
1
0
1
10
100
Cohort 1
Cohort 2
B
Peripheral Blood, Post-Exposure
64
32
16
8
4
2
1
C
Bone Marrow, Post-Exposure

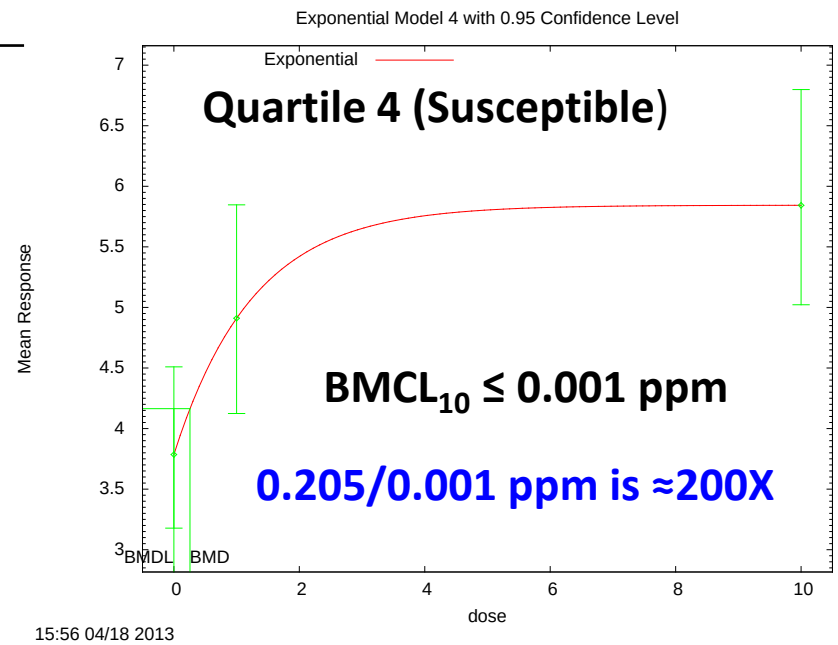


Benchmark Concentration Models
DO vs. B6C3F1 (Farris et al. 1996)

Quartile	BMC	$BMCL_{10}$
All quartiles	0.367	0.200
Q1	0.550	0.205
Q2+Q3	0.315	0.117
Q2+Q3+Q4	0.275	0.130
Q4	0.257	<0.001







Benchmark Concentration Model (BMC)

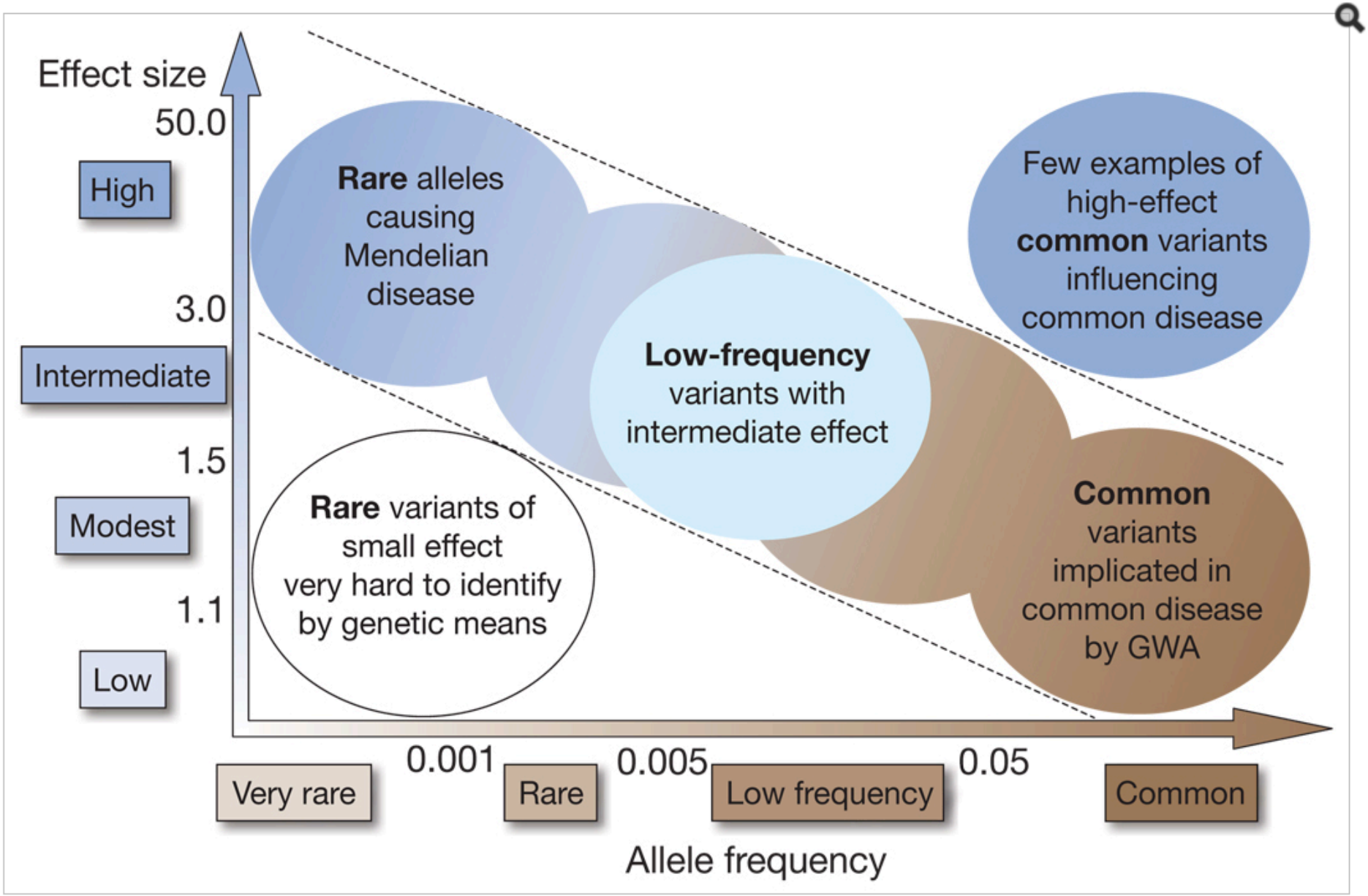
EPA Risk Assessment 1988 (Updated & revised 2003)

BMCL* = 8.2 mg/m^3 (2.6 ppm); UF = 300; MF = 1

RfC = 3 x 10^{-2} mg/m^3 (9.4 ppb)**

*Decreased lymphocyte count in 44 individuals **(Rothman et al., Am. J. Ind. Med. 29:236,1996**). No metric provide (i.e. 1SD or 10% above the control mean).

***No adverse effect level peripheral blood HSC counts determined in occupational exposures to benzene (Lan et al. Science 306:1774, 2004 & McHale et al. 33:240, 2012)**



Feasibility of identifying genetic variants by risk allele frequency and strength of genetic effect (odds ratio)

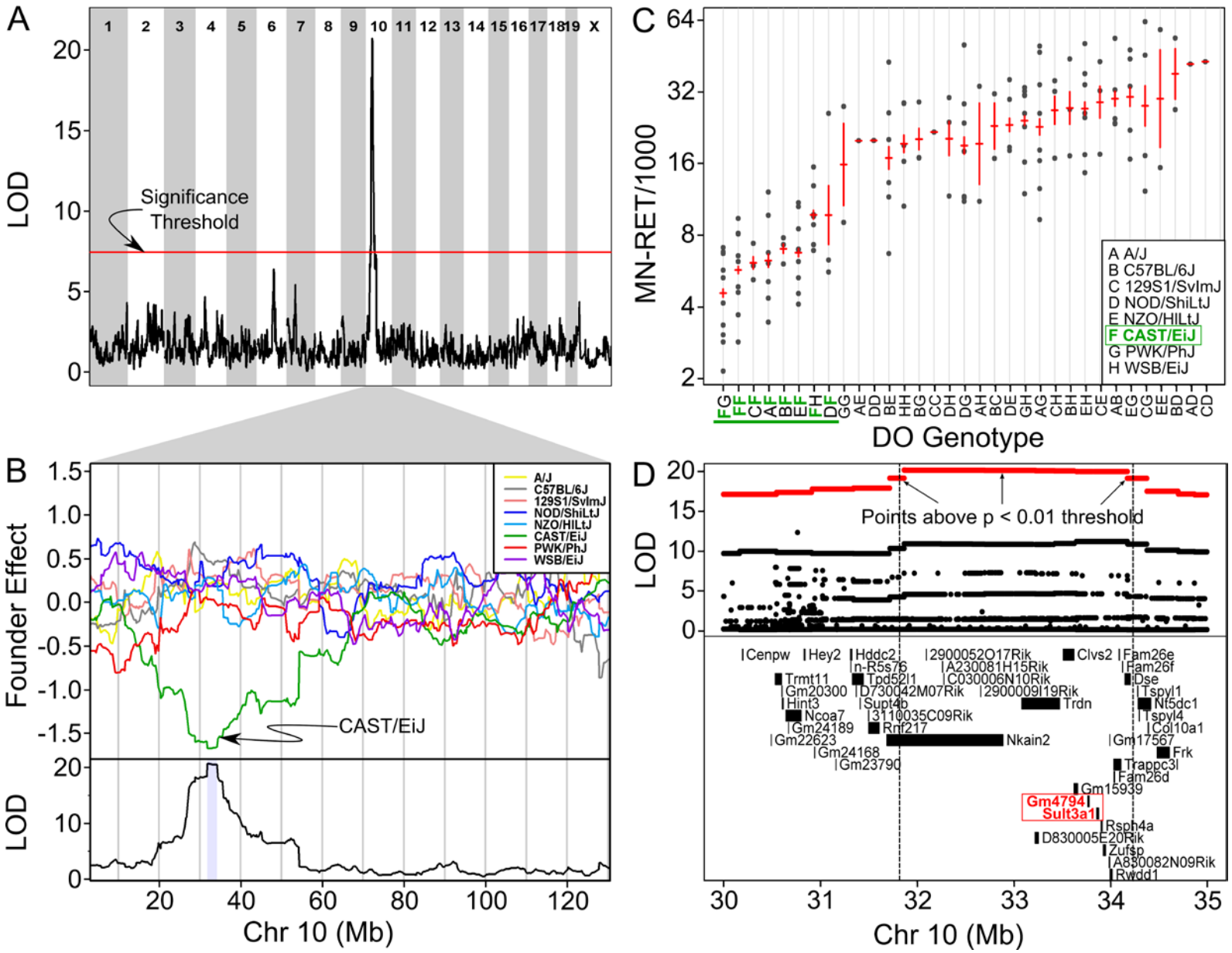
Manolio et al. Nature 462:747 (2009) and McCarthy et al. Hum Mol Gen 17:R156 (2008)

DO mouse population linkage mapping

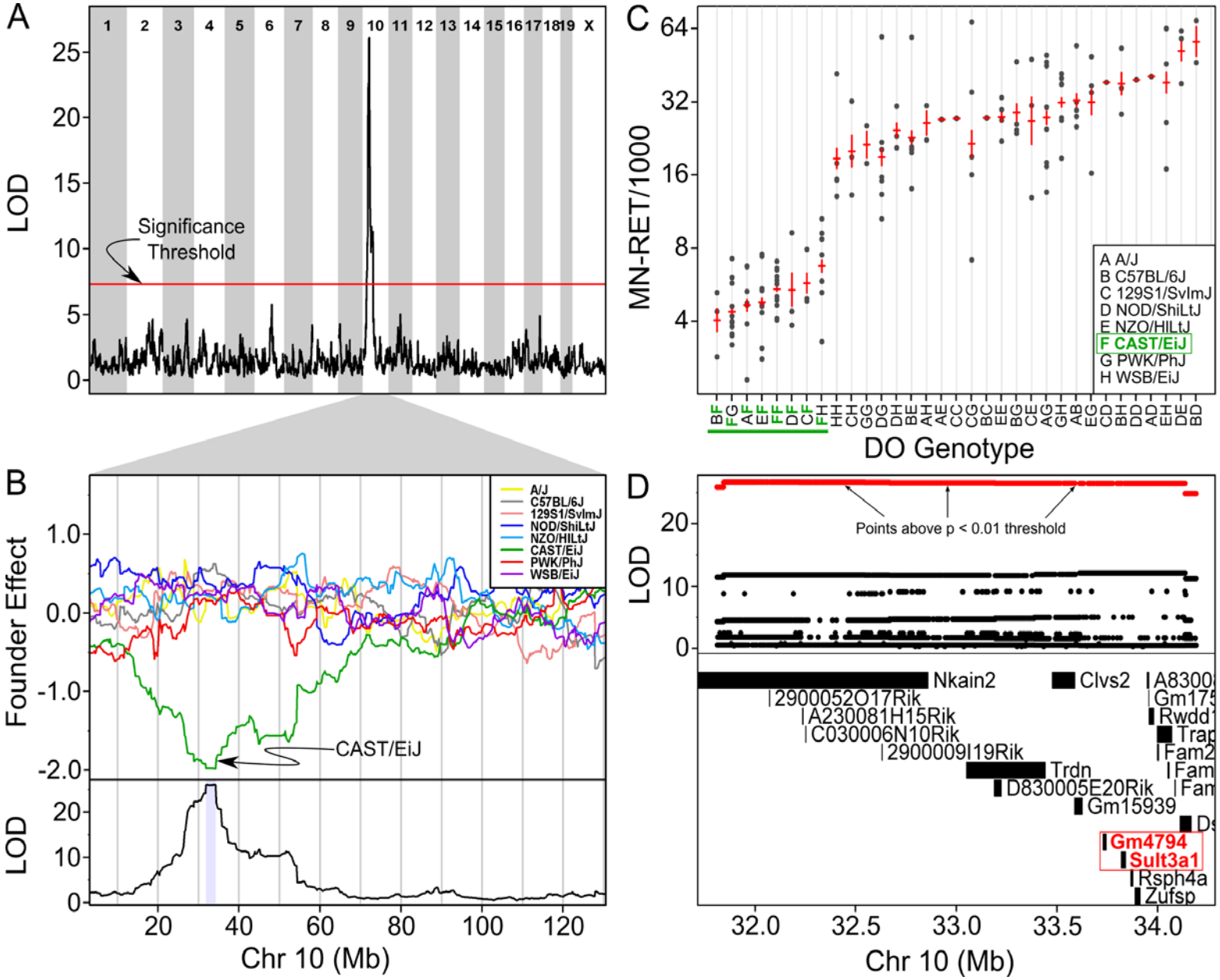
- Genetically heterogeneous reference population
- Derived from a set of 8 inbred founder strains (from the Collaborative Cross)
- 45 million segregating SNPs, indels, & CNV
- Balanced founder allele (1/8) frequencies averaging 12%
- Combination of high genetic diversity, low MAF, and fine recombination block structure make the DO mice ideal for genetic mapping

Svenson KL et al. High-resolution genetic mapping using the Mouse Diversity outbred population. Genetics 2012;190:437-47.

Bone Marrow MN-RET/1000RET

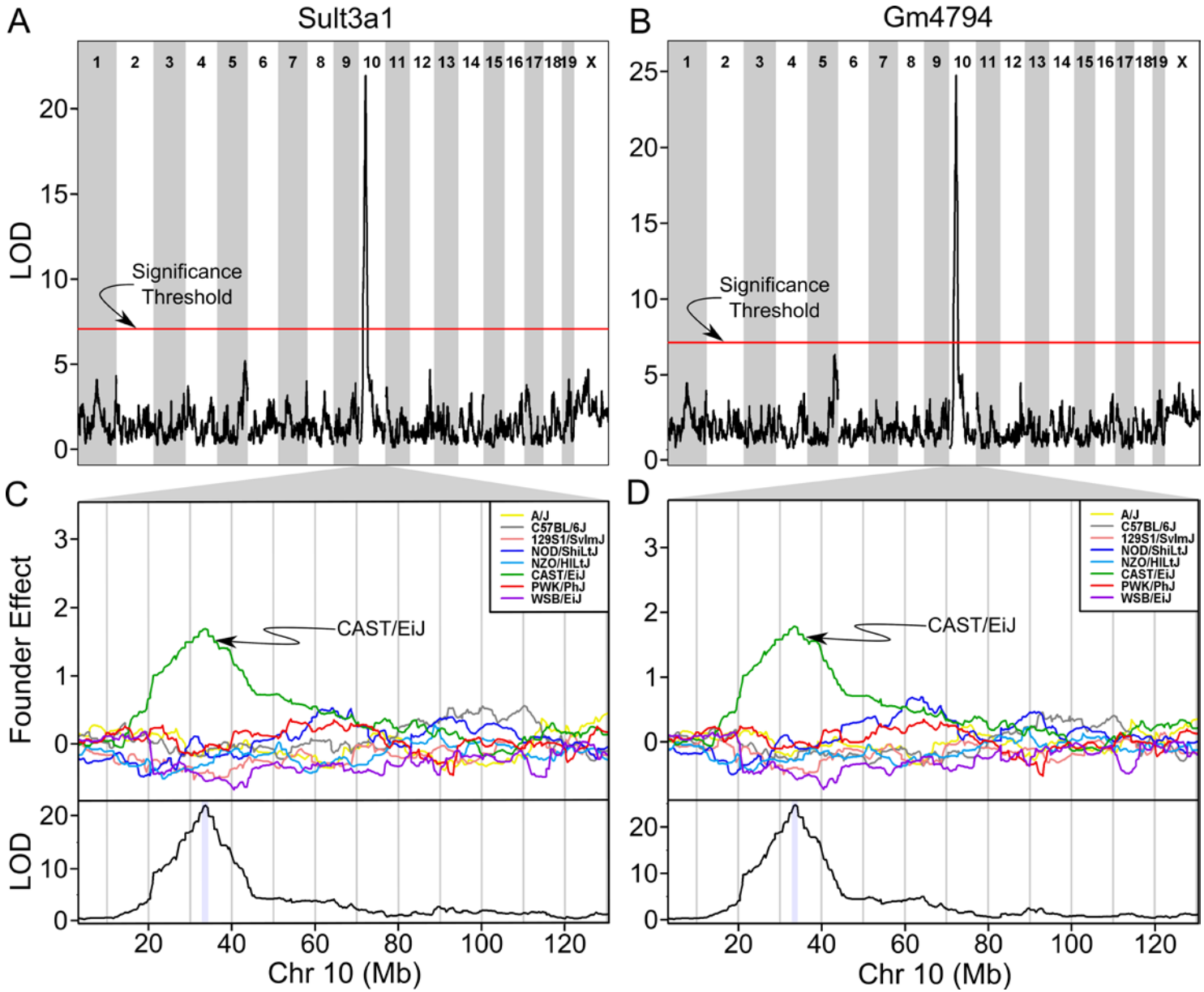


Peripheral Blood MN-RET/1000RET

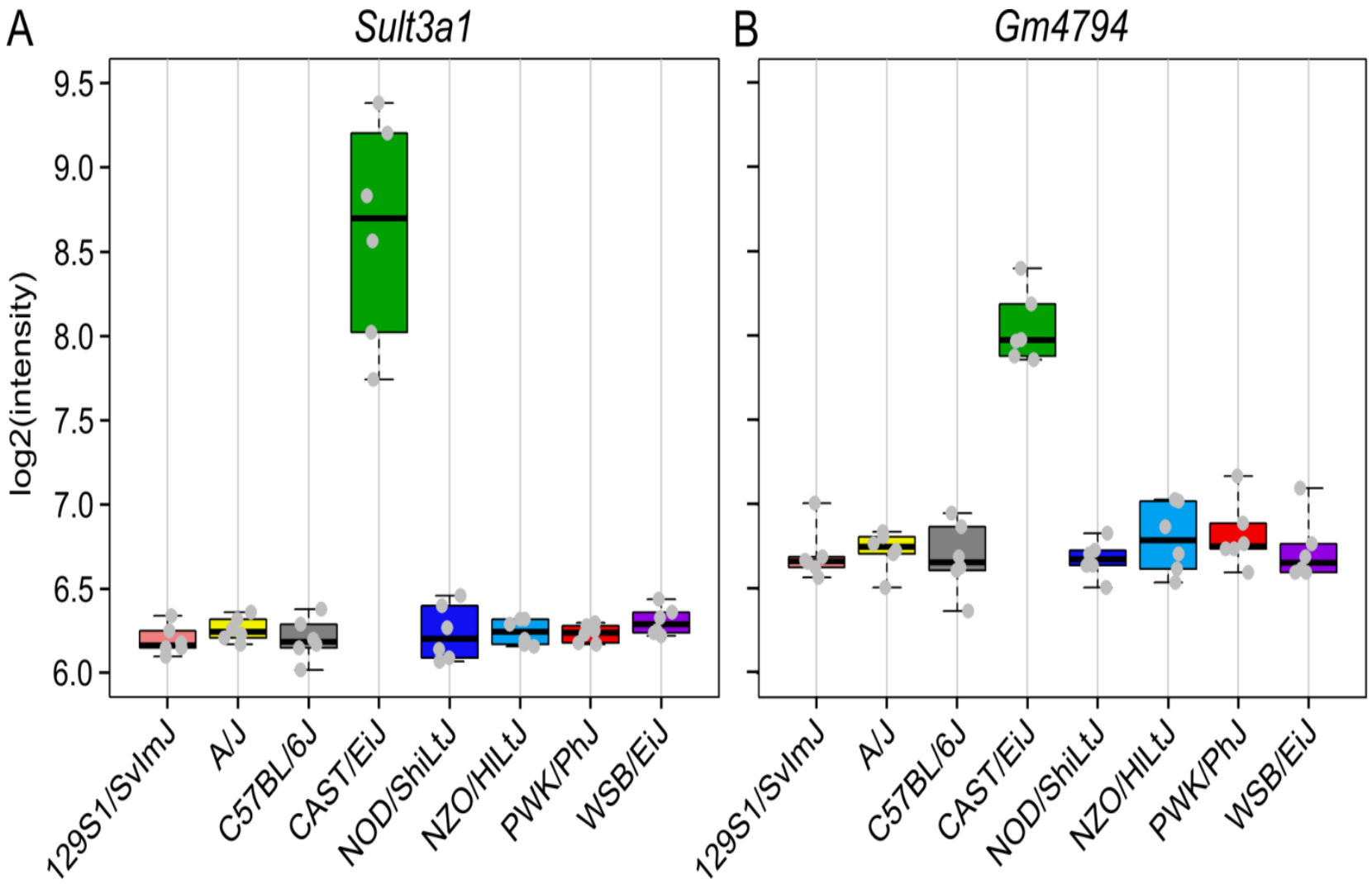


Gene symbol	Name / description	Chr location (GRCm	Size	Alert/ Location	Identifier
Nkain2	Na+/K+ transporting ATPase interacting 2	10:31689319-32889915 (–)	1200596	Structural variant* Ins 31838075-31838077 del 32310195-32319195	MGI:1923447
Trdn	Triadin	10:33083483-33476709 (+)	393226	Nonsynonymous SNP* rs33783582 rs33784331 Structural variant del 33269490-33269848	MGI:1924007
Clvs2	Clavesin 2	10:33512286-33624769 (–)	112483	Structural variant del 33578793-33578899	MGI:2443223
Gm4794	**Predicted gene 4794**	**10:33766424-337821**	**15691**	**Structural variant del 33772397-33777130 del 33779262-33779648**	**MGI:3645972**
Sult3a1	**Sulfotransferase family 3A, member 1**	**10:33863935-338794**	**15540**	**Structural variant gain 112K bp 33876195**	**MGI:1931469**
Rsph4a	Radial spoke head 4 homolog A	10:33905485-33915883 (+)	10398	Nonsynonymous SNP rs253783385 rs33802524	MGI:3027894
Zufsp	Zinc finger with UFM1 specific peptidase domain	10:33926936-33951212 (–)	24276	Nonsynonymous SNP* rs48254962	MGI:1919830

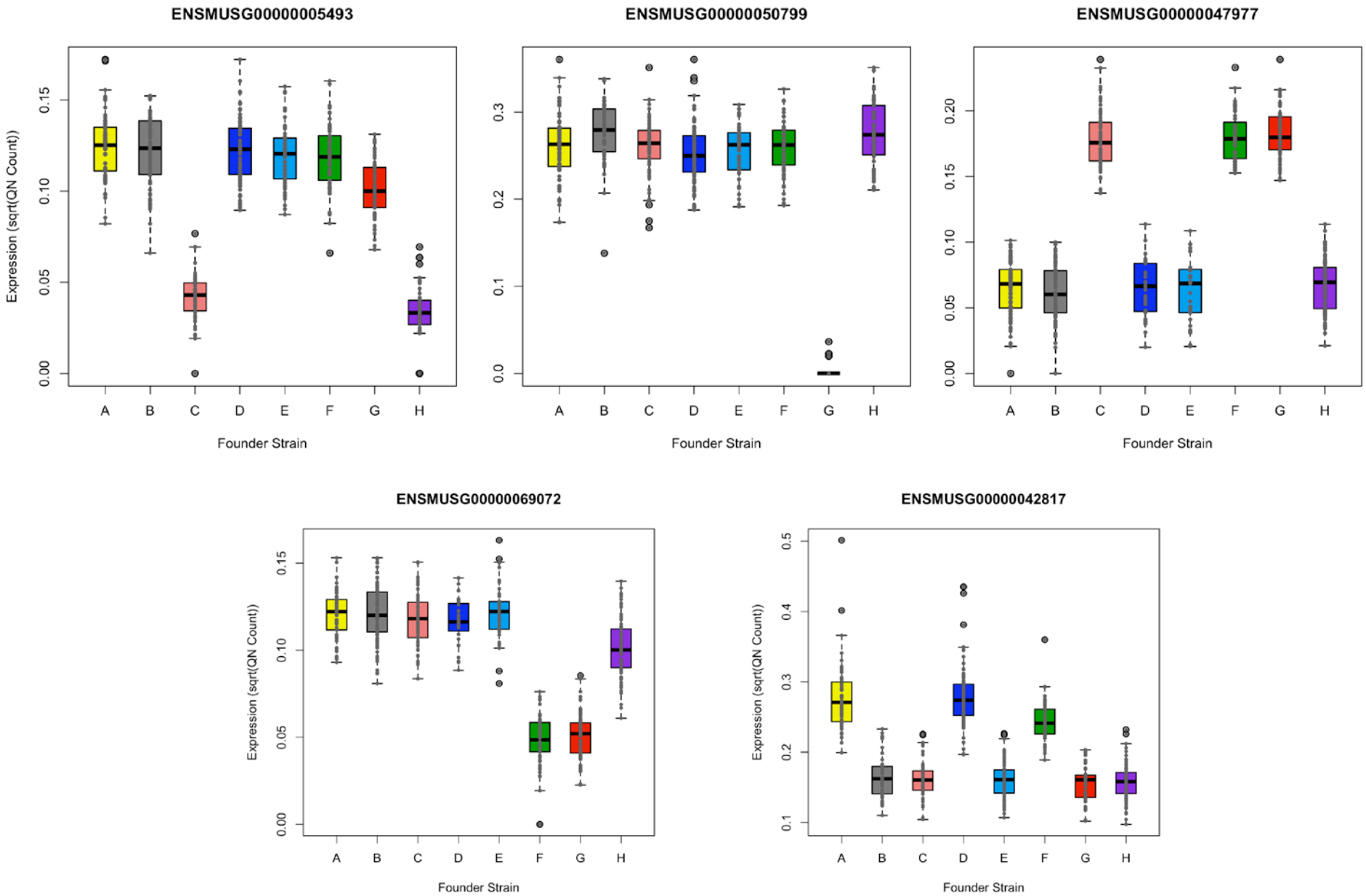
Expression QTL in DO mice (Liver)



Homozygous inbred strains mRNA liver expression levels



Allele specific expression is the rule



Human copy number & 5mCpG variation

- *SULT1A1 phenol sulfotransferase*

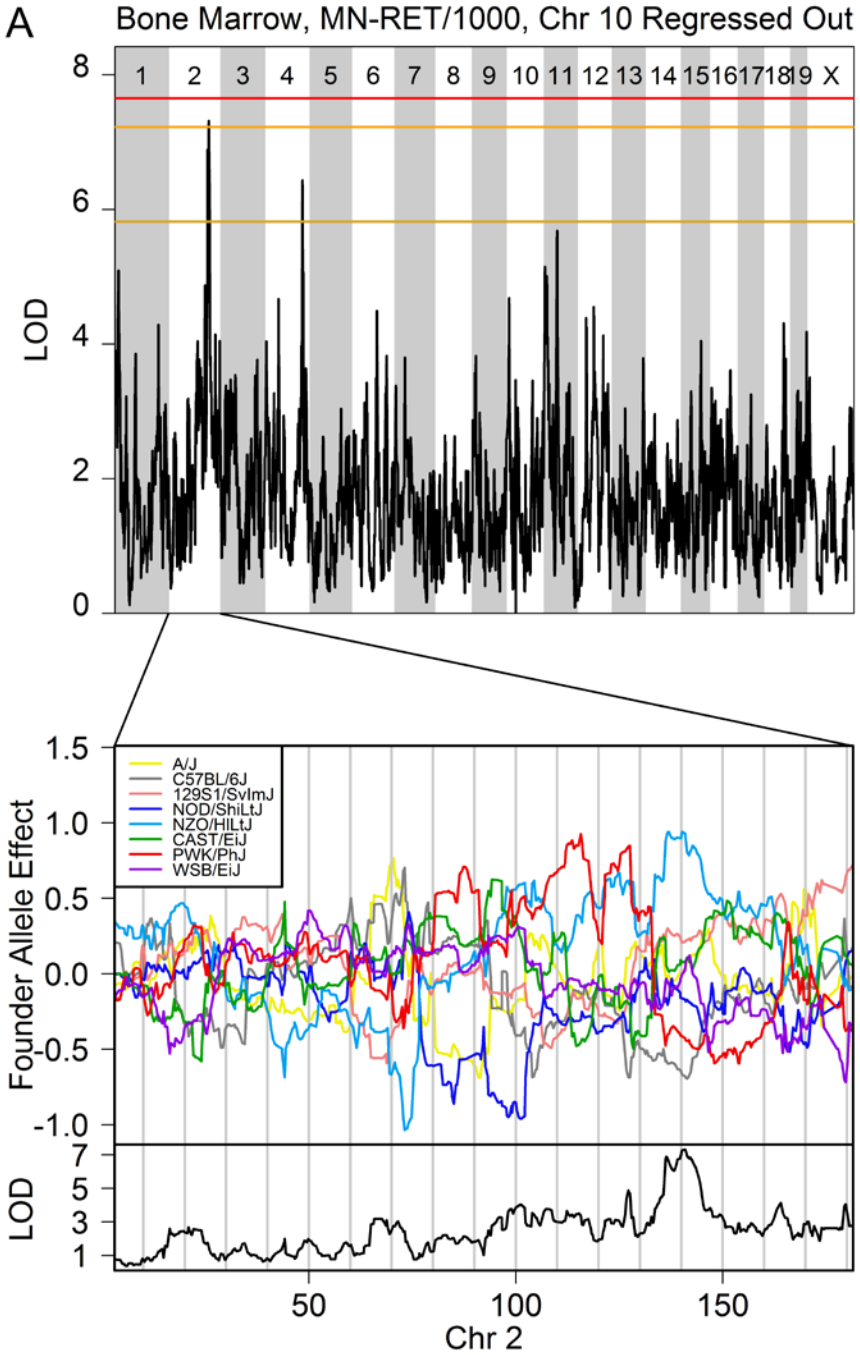
CNV range 1-6 copies*

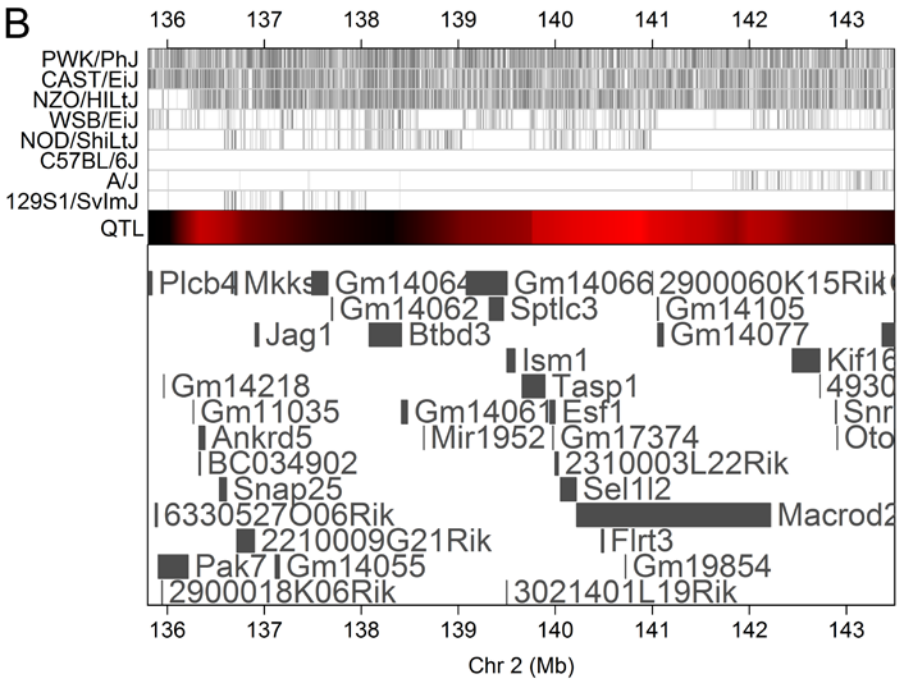
Caucasian: 5% -single copy; 69% - 2 copies; 26% -3 or more copies (**362 individuals**)

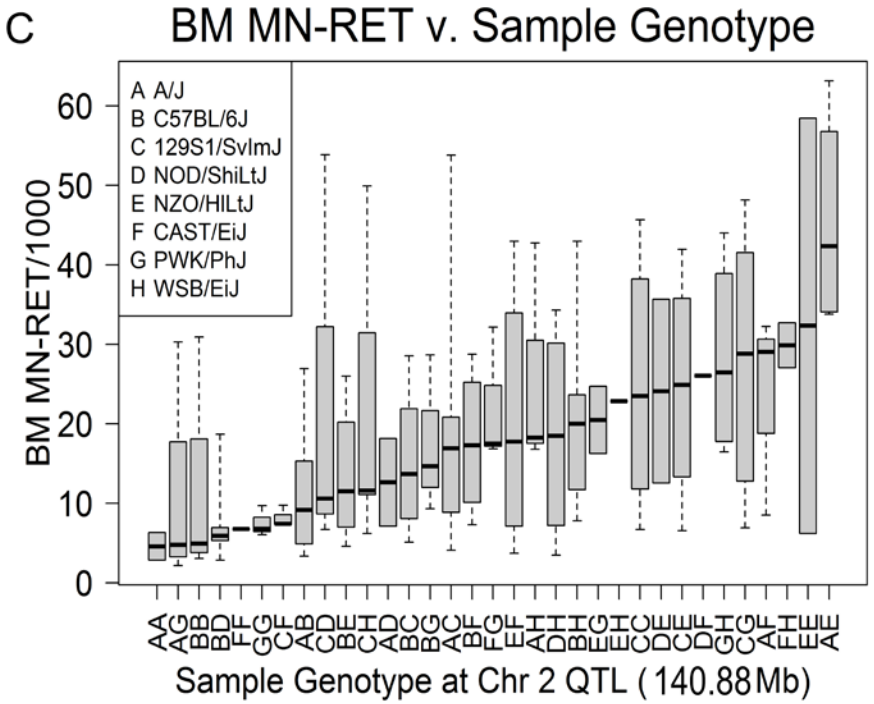
African-Americans: 0% - single copy; 38% - 2 copies; 62% - 3 or more copies (**99 individuals**)

Hebbring et al. Human Molecular Genetics 16, 463, 2007

Gaedigk et al. Pharmacogenomics 13, 91, 2012


A
Bone Marrow, MN-RET/1000, Chr 10 Regressed Out
LOD
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 X
A/J
C57BL/6J
129S1/SvImJ
NOD/ShiLtJ
NZO/HILtJ
CAST/EiJ
PWK/PhJ
WSB/EiJ
Founder Allele Effect
LOD
Chr 2


B
PWK/PhJ
CAST/EiJ
NZO/HILtJ
WSB/EiJ
NOD/ShiLtJ
C57BL/6J
A/J
129S1/SvImJ
QTL
Plcb4
Mkks
Gm14064
Gm14066
2900060K15Rik
Gm14062
Sptlc3
Gm14105
Jag1
Btbd3
Gm14077
Ism1
Kif16
Gm14218
Tasp1
4930
Gm11035
Gm14061
Esf1
Snr
Ankrd5
Mir1952
Gm17374
Oto
BC034902
2310003L22Rik
Snap25
Sel1l2
6330527O06Rik
Macrod2
2210009G21Rik
Flrt3
Pak7
Gm14055
Gm19854
2900018K06Rik
3021401L19Rik
Chr 2 (Mb)


C
BM MN-RET v. Sample Genotype
A A/J
B C57BL/6J
C 129S1/SvImJ
D NOD/ShiLtJ
E NZO/HILtJ
F CAST/EiJ
G PWK/PhJ
H WSB/EiJ
BM MN-RET/1000
Sample Genotype at Chr 2 QTL (140.88 Mb)

Gene symbol	Name / description	Chr location (GRCm38)	Size	Alert	Identifier
Gm14066	Predicted gene 14066	2:139256876-139677714 –	420838	Structural Variant BP139642997	MGI:3649728
Sptlc3	Serine palmitoyltransferase, long chain base subunit 3	2:139493913-139637674 +	143761	Structural Variant BP139503281 BP139503283	MGI:2444678
Tasp1	Taspase, threonine aspartase 1	2:139833480-140066805 –	233325	Structural Variant* BP139862837 BP139866448	MGI:1923062
Esf1	ESF1, nucleolar pre-rRNA processing protein	2:140119883-140170564 –	50681	Nonsynonymous SNP rs29958536	MGI:1913830
Macrod2	**MACRO domain containing 2**	**2:140395309-142392966 +**	**1997657**	**Structural Variant* BP140551698 BP140551700**	**MGI:1920149**
Gm14077	Predicted gene 14077	2:141230431-141286991 –	56560	Structural Variant BP141233538	MGI:3650192
Kif16b	**Kinesin family member 16B**	**2:142617474-142901531 –**	**284057**	**Nonsynonymous SNP rs27214196**	**MGI:109824**

Summary & Conclusions

- DO mice show significant population variance
- Significant linkage mapping power
- Reproducibility of outcome (2 cohorts)
- MN-RET reliable quantitative biomarker
- Identification of individual DO mouse resistance and susceptibility genotypes
- Potential to inform genetic epidemiology studies and provide statistical evidence for identifying genes with significant effect size & hypothesis based research

Possible directions

- Repeat BMC modeling with additional exposures at 0.1 ppm and 50 ppm to better define shape of the dose response curve
- Perform 2 year inhalation exposure at 0.1, 1, and 10 ppm to define cancer BMCL and tumor phenotypes
- Stratify BMCL by quartile or decile to estimate the most susceptible subpopulation at risk

Funding

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Next steps and future research…..

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