Reverse Translational Studies to Understand Drug-Induced Toxicity

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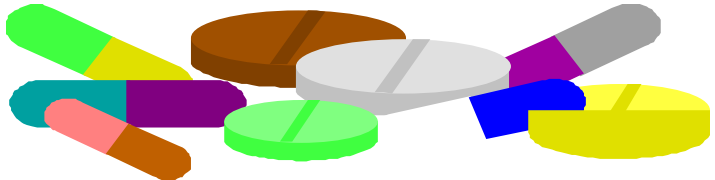
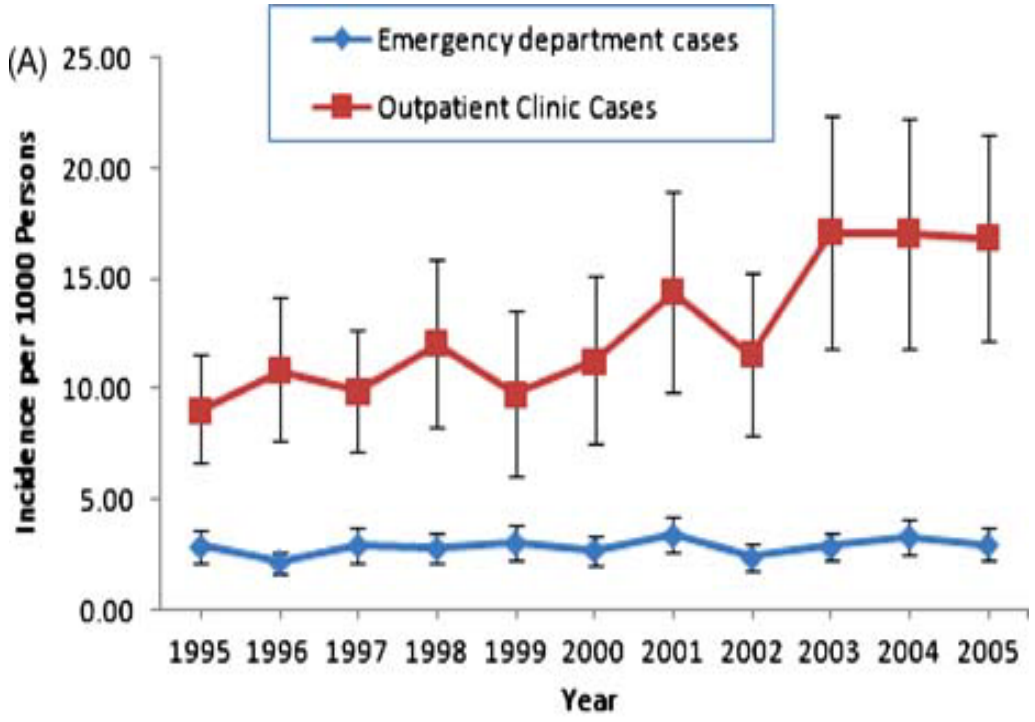
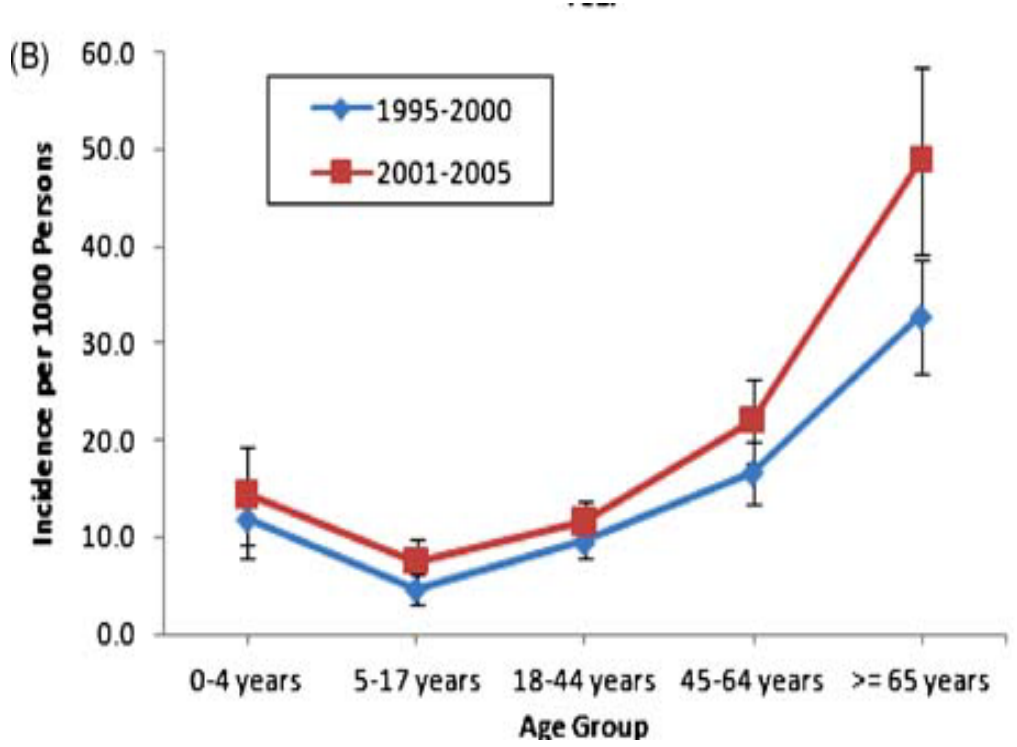


Adverse Drug Events Are Significant Clinical Problems

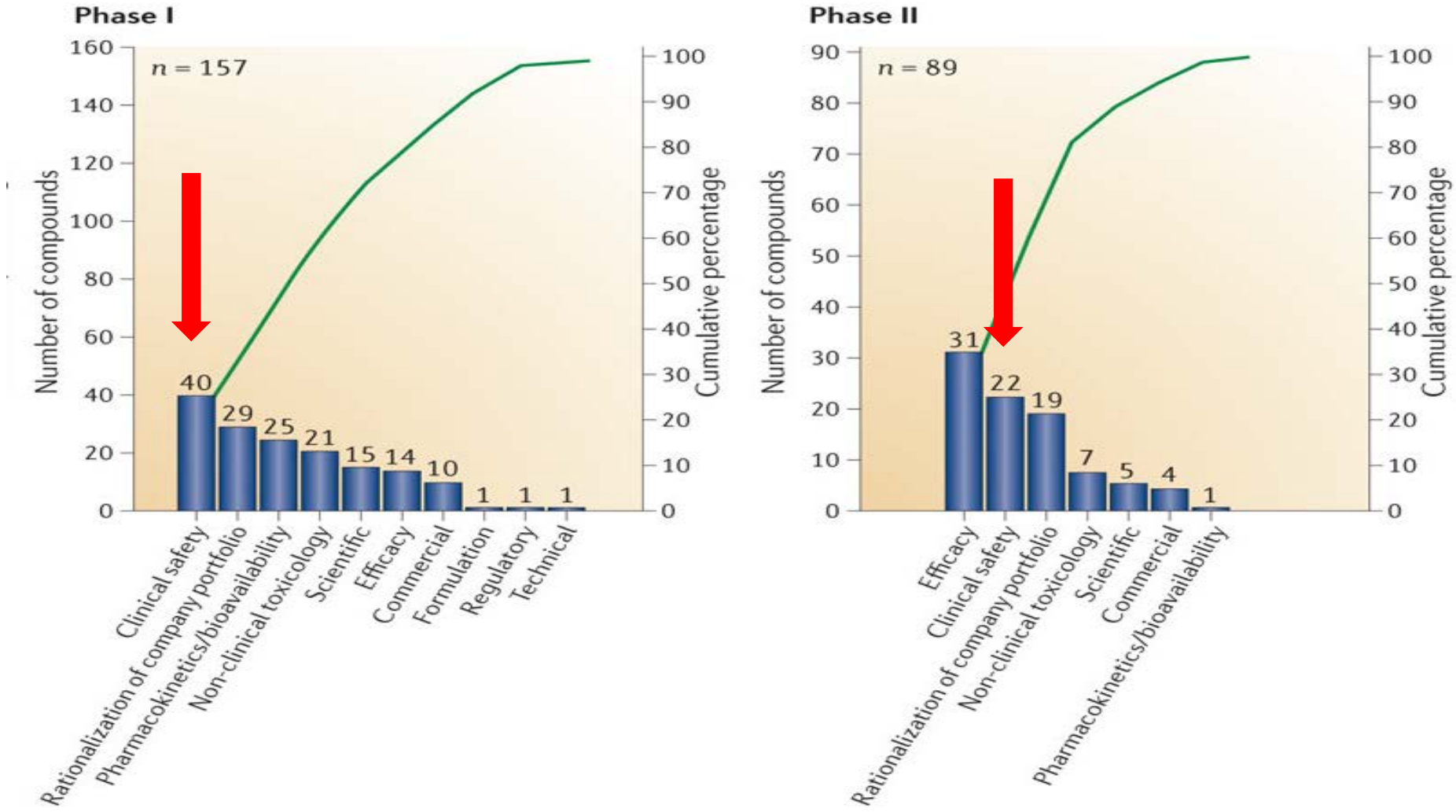
~4.5 million adverse drug event-related visits to clinic or ER/yr





Bourgeois et al. *Pharmacoepi Drug Safety* 19:901 (2010)

Attrition Due to Clinical Safety Impedes New Drug Development



Waring et al. *Nature Rev Drug Discovery* 14:475 (2015)

Nature Reviews | Drug Discovery

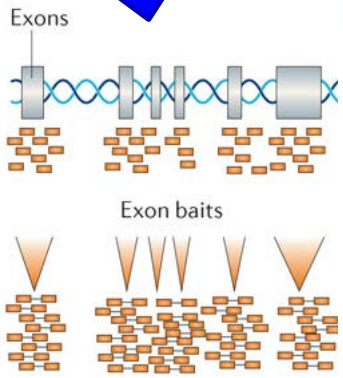
A Major Goal of the Precision Medicine Initiative: Reduce Therapeutic Adverse Events

- Precision Medicine (2015)
  - Tailoring of medical treatment to individual characteristics such as lifestyle, environmental and biological uniqueness (i.e., genome, microbiome, etc.)
- Goal
  - Focusing therapeutic interventions on those who would benefit
  - ***Sparing expense and adverse events for those who will not***
- Driver
  - Advances in technology

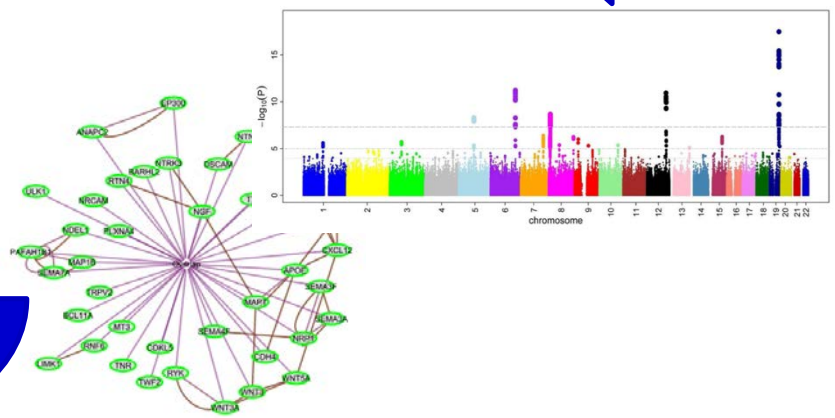
All of Us℠ The Precision Medicine Initiative®
THE FUTURE OF HEALTH BEGINS WITH YOU

Reverse Translation of Adverse Drug Events

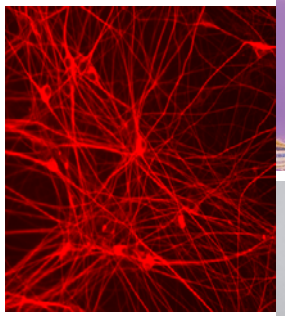
Bedside to Bench



Computational Analysis



Lab Analyses



Bench to Bedside

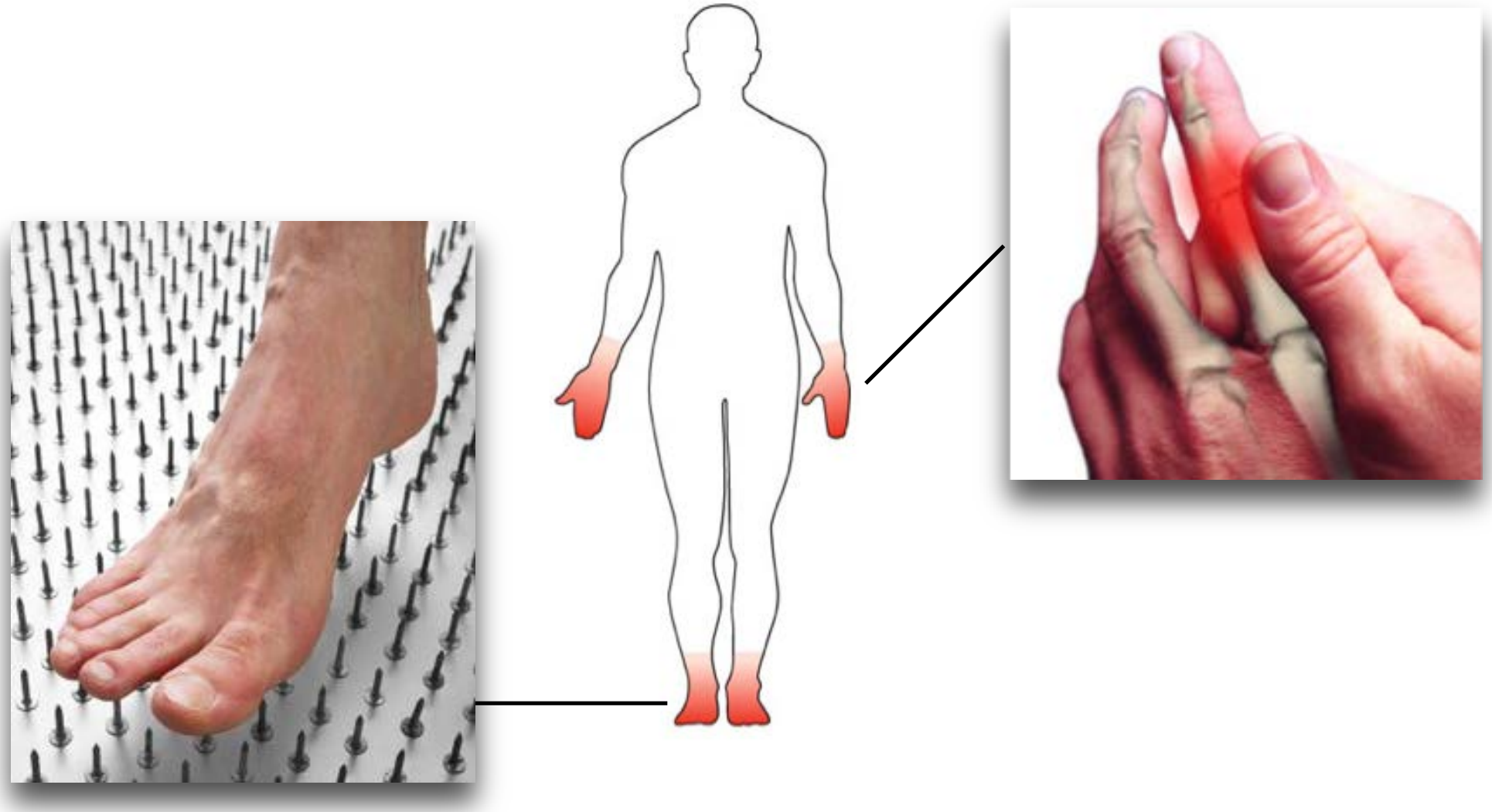
Motivation

- Improved patient outcomes
- Increased understanding of molecular mechanisms for drug toxicities
    - o Targeted therapies to treat/prevent toxicity
    - o Screening in drug development

Two Tales of Reverse Translation: From Genomics towards Mechanism

- Chemotherapy-induced peripheral neuropathy
  - GWAS
  - iPSC-Induced sensory neuron studies
- Bevacizumab-induced hypertension
  - Exome sequencing
  - Cell-based studies

Sensory Peripheral Neuropathy is a Serious and Common Adverse Event



Chemotherapy-Induced Sensory Peripheral Neuropathy (CIPN)

- Affects 30-40% of cancer patients
  - Platinum agents, **Taxanes, Epothilones, Eribulin**, Vincristine, Bortezomib, Thalidomide, Lenalidomide
- One of the most common reasons that cancer patients stop treatment early
- Affects quality of life
- No effective drug therapies to prevent CIPN
- Largely managed with physical therapy, massage, accupuncture

Despite Decades of Animal Studies There are No Effective Strategies for Prevention of CIPN

Strategy	ASCO Recommendation		
	Strong Against	Moderate Against	Inconclusive
Neuroprotectants	Acetyl-L-carnitine Diethyldithiocarbamate	Amifostine Leukemia Inhibitory Factor ACTH analogs	Glutamine/Glutamate
Neurotransmitter Release		Amitryptyline	Venlafaxine
Channel activity	Nimodipine	Ca^{2+}/Mg^{2+}	Carbamazepine Oxcarbazepine
Antioxidants	Acetyl-L-carnitine	Vitamin E GSH Retinoic Acid	N-acetylcysteine ω3 Fatty Acids

Hershman et al. *J Clin Oncol* 32:1941-1967 (2014)

And Only Moderate Evidence for Effective Treatment of CIPN

Strategy	ASCO Recommendation		
	Moderate For	Moderate Against	Inconclusive
Neuroprotectants			Acetyl-L-carnitine
Neurotransmitter Release	Duloxetine		Nortriptyline/Amitryptyline Topical Amitriptyline/Ketamine/ ±Baclofen
Channel Activity		Lamotrigine	Gabapentin

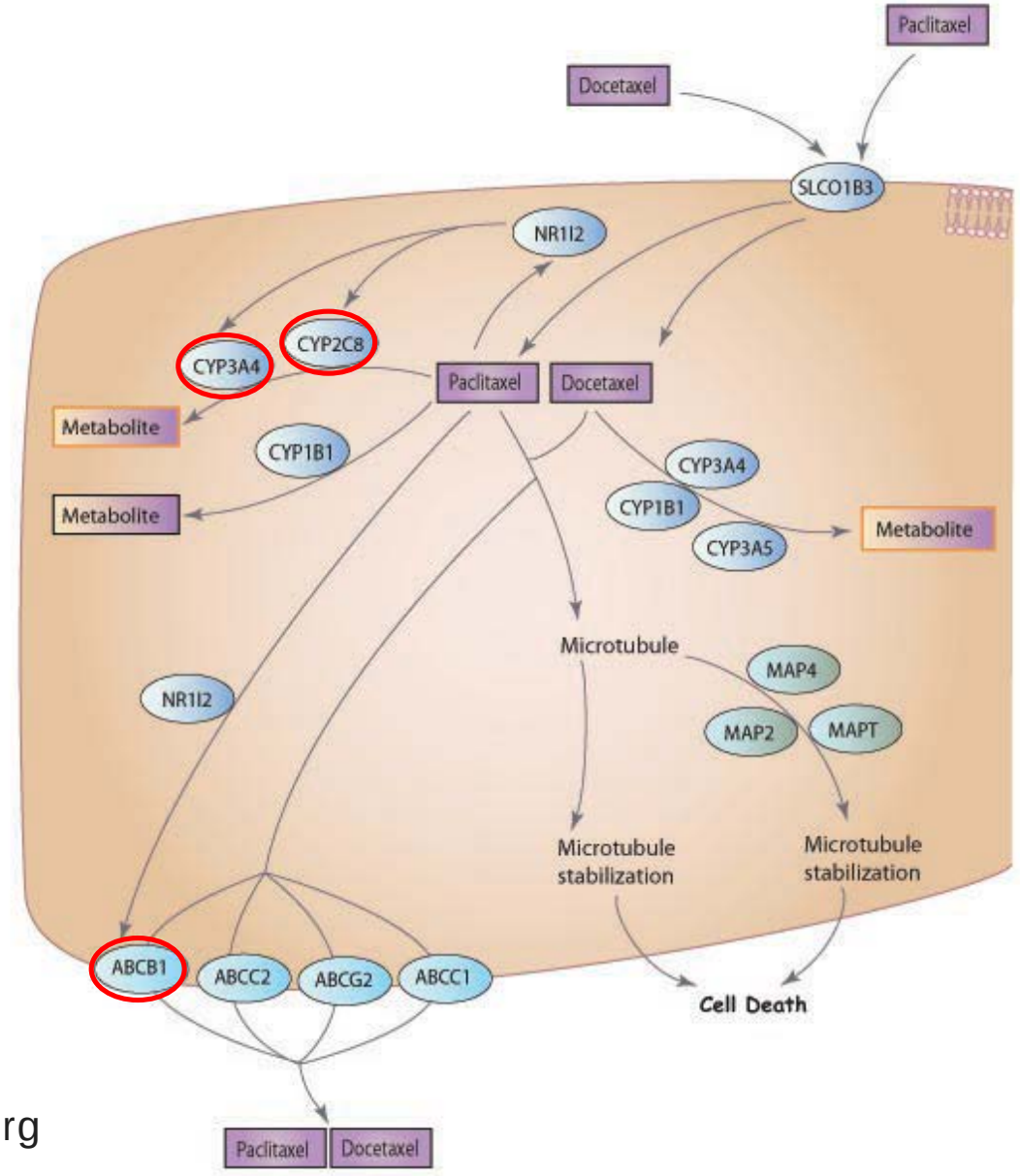
Hershman et al. *J Clin Oncol* 32:1941-1967 (2014)

Paclitaxel-Induced Sensory Peripheral Neuropathy

- Risk factors include:
  - high single dose
  - high cumulative dose
  - treatment with other neurotoxic drugs
  - other conditions which cause neuropathy (*e.g.*, diabetes, alcoholism, HIV)

- Genetic Variability?

Candidate Gene Studies Focused on Taxane Response Pathway



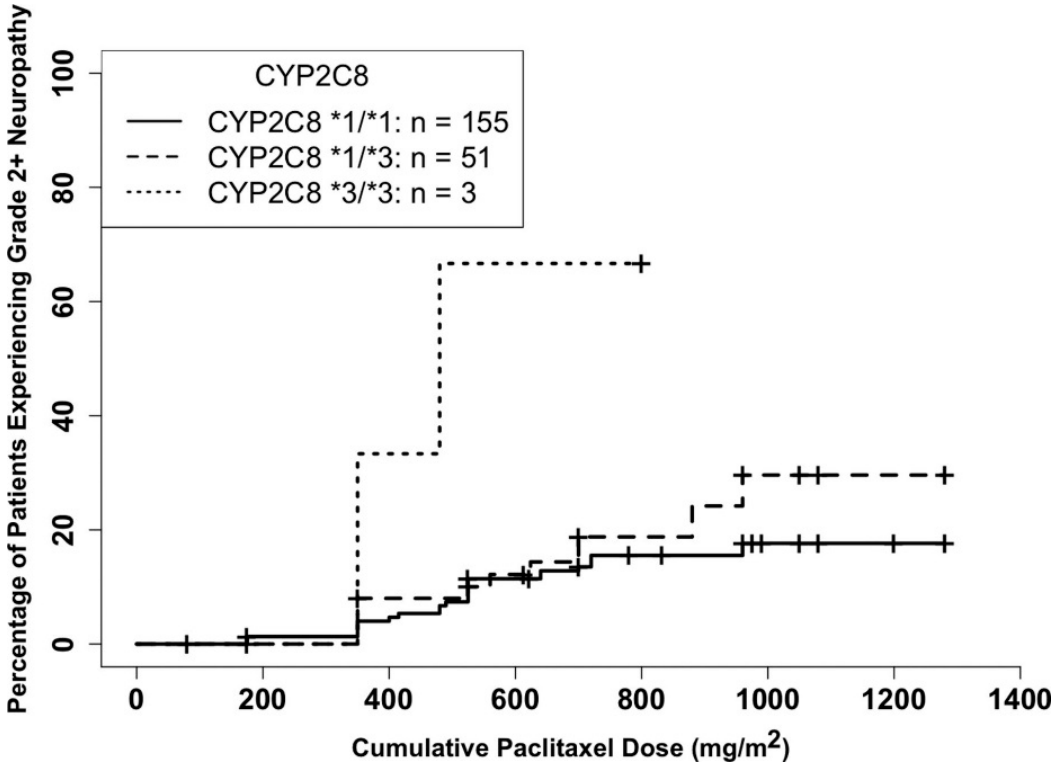
Most associations had small effect sizes and did not consistently replicate in additional studies

www.pharmagkb.org

Cliff et al. *Crit Rev Oncol Hematol* 120: 127 (2017)

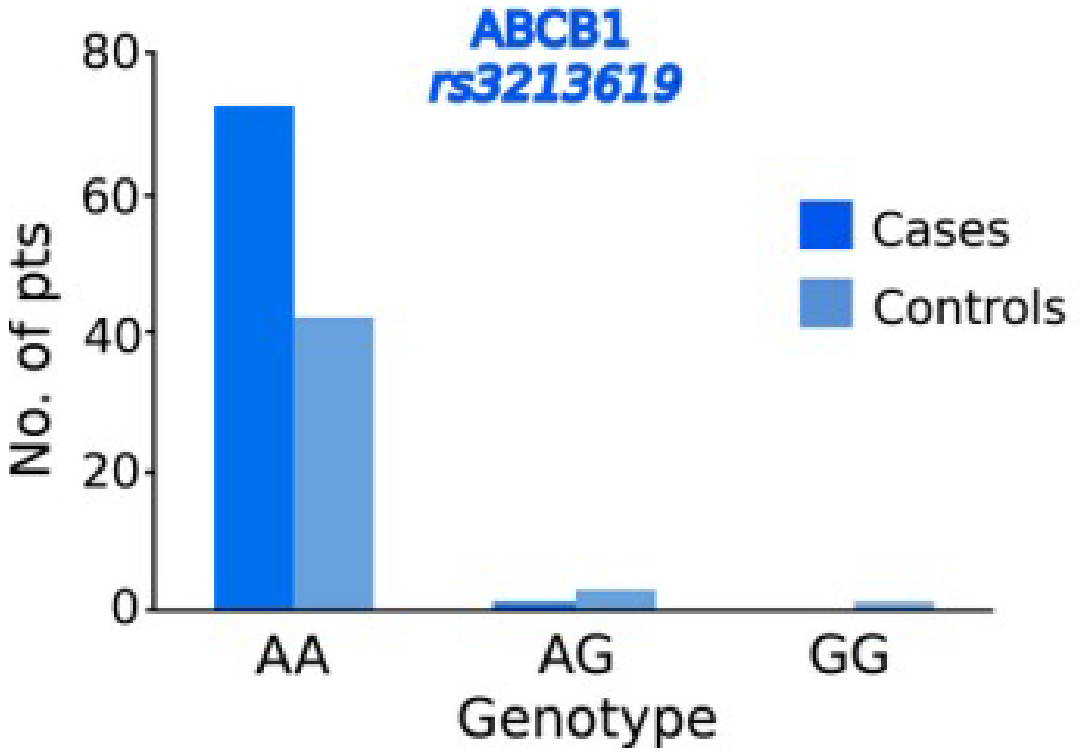
Association of *CYP2C8*3* and *ABCB1* -129A>G with Paclitaxel-Induced Neuropathy

Grade 2+
HR = 1.95 (1.06 – 3.58)
***P* = 0.031**



Hertz D L et al. *Ann Oncol* 24:1472-1478 (2013)

Extreme phenotyping
73 cases, 46 controls
HR = 0.12
***P* = 0.03**

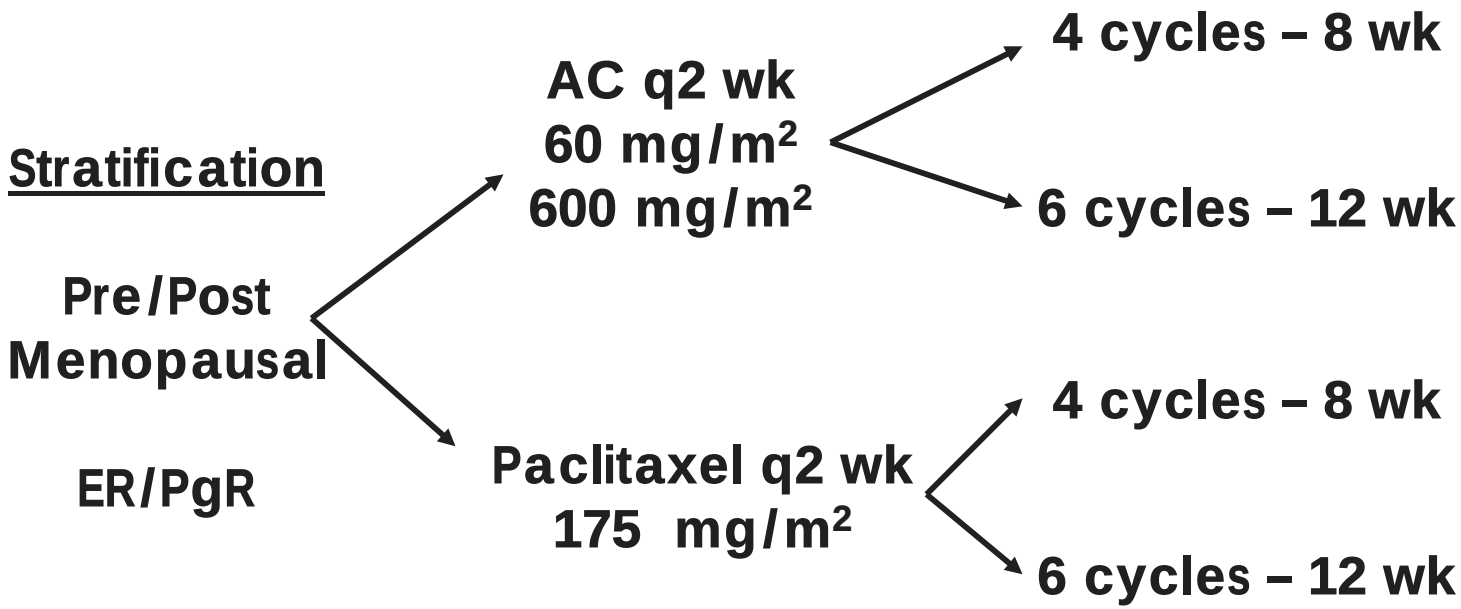


Boora et al. *Cancer Med* 5:631-639 (2016)

CALGB/Alliance Microtubule Targeting Agent Breast Cancer Studies with Pharmacogenetic Companions

Study	Drugs	PG Samples	Phenotypes	Genotyping
40101/60202 (Shulman/ Kroetz)	Paclitaxel Adriamycin/ cyclophosphamide	2294	Peripheral neuropathy Neutropenia Ovarian suppression Cardiotoxicity	GWAS, targeted resequencing
40502/60704 (Rugo/ Kroetz)	Paclitaxel Nab-paclitaxel Ixabepilone Bevacizumab	621	Peripheral neuropathy Response Hypertension	GWAS, Exome sequencing
40601/60701 (Carey/ Kroetz)	Paclitaxel Trastuzumab Lapatanib	211	Response Peripheral neuropathy Cardiotoxicity	Replication for exome sequencing
40603/60703 (Sikov/ Kroetz)	Paclitaxel Carboplatin Bevacizumab	379	Response Peripheral neuropathy Hypertension	Exome sequencing

CALGB 40101 – 2 X 2 Factorial Design
Adjuvant Therapy for Women with Breast Cancer with 0-3 + Nodes

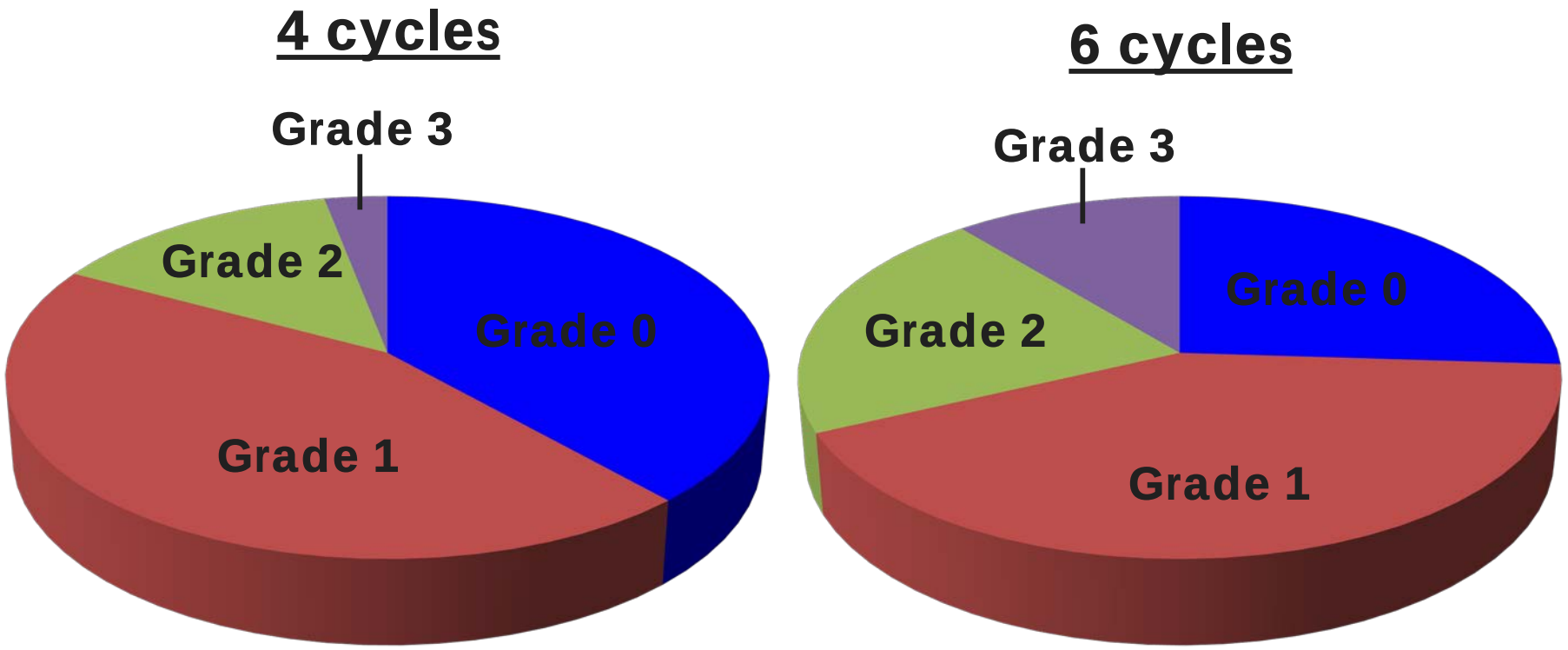


AC = doxorubicin/cyclophosphamide

Total Accrual = 3871
Accrual to Paclitaxel 4 cycles = 1005
Accrual to Paclitaxel 6 cycles = 648

Shulman et al. *Clin Cancer Res* 32:2311-2317 (2014)

CALGB 40101: Paclitaxel-Induced Sensory Peripheral Neuropathy is Dose Dependent

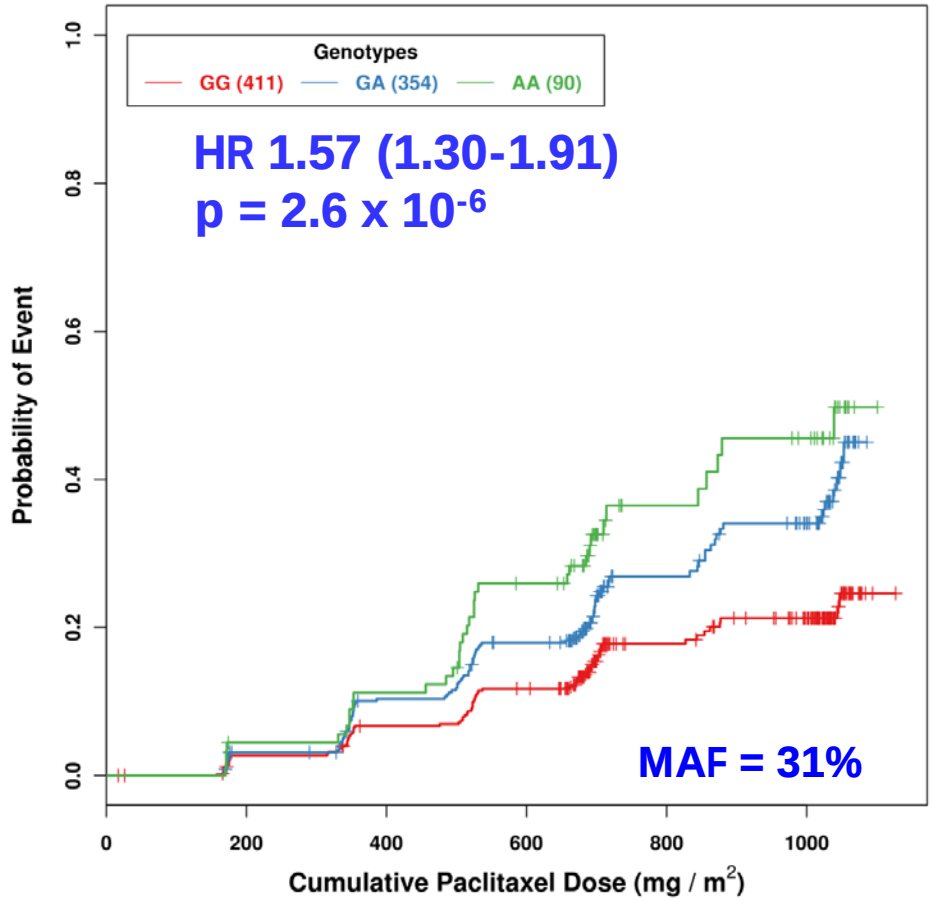


- Overall incidence of ≥ Grade 2 sensory peripheral neuropathy was 24%
  - 17% in 4 cycle arm
  - 33% in 6 cycle arm

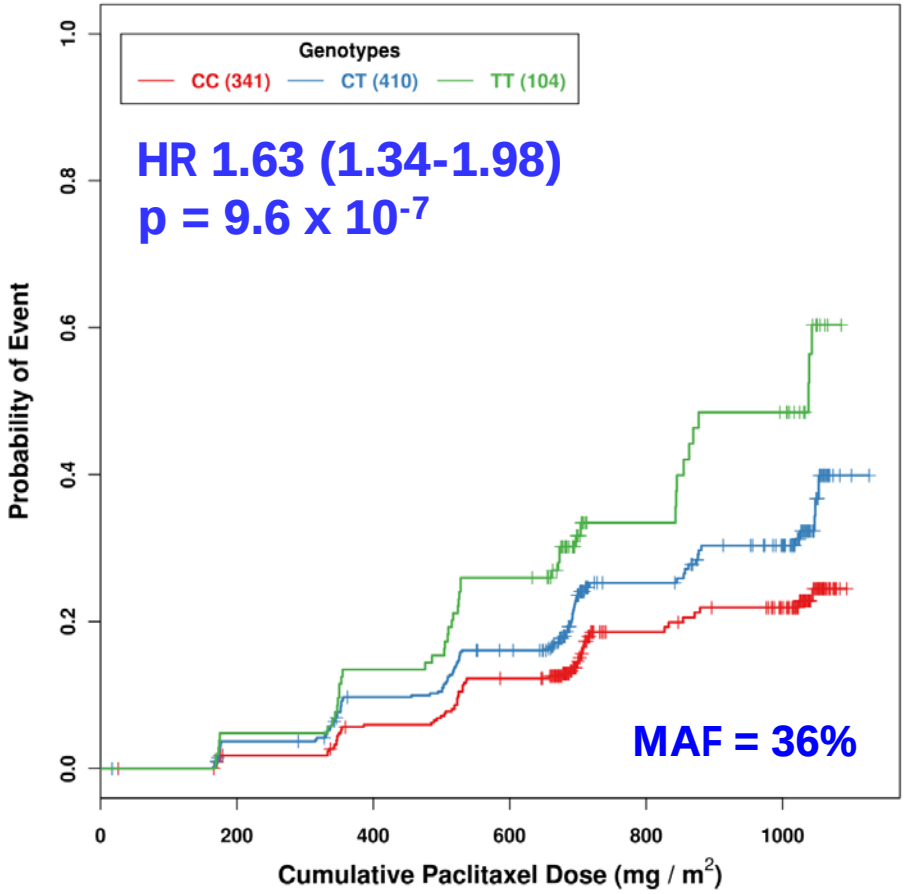
Common *FGD4* and *EPHA5* SNPs Associated with Onset of Sensory Peripheral Neuropathy

N = 855

FGD4 - rs10771973



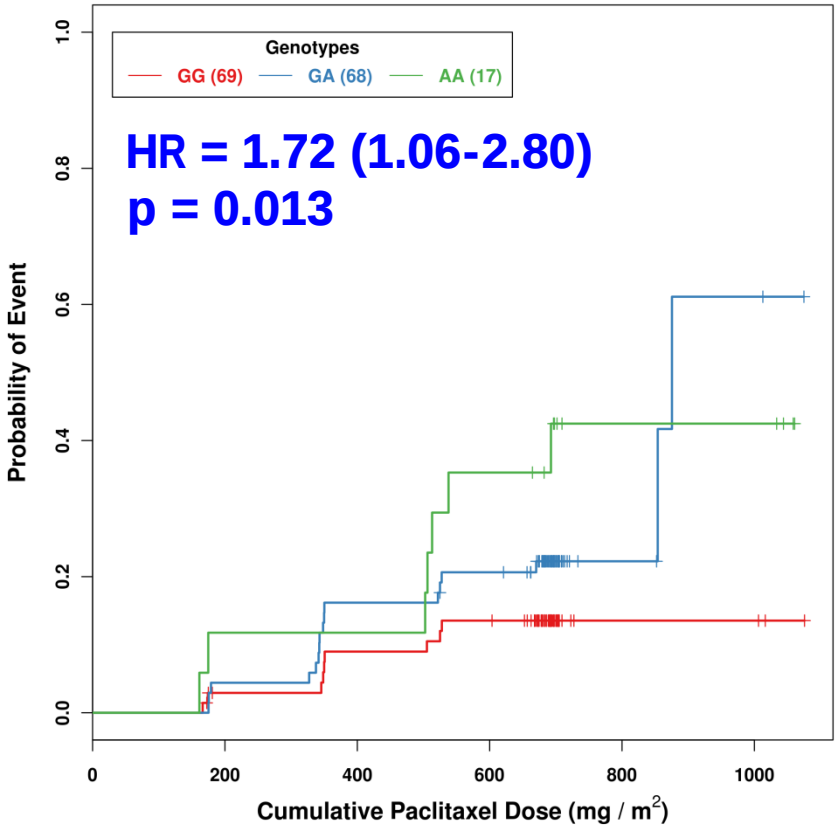
EPHA5 - rs7349683



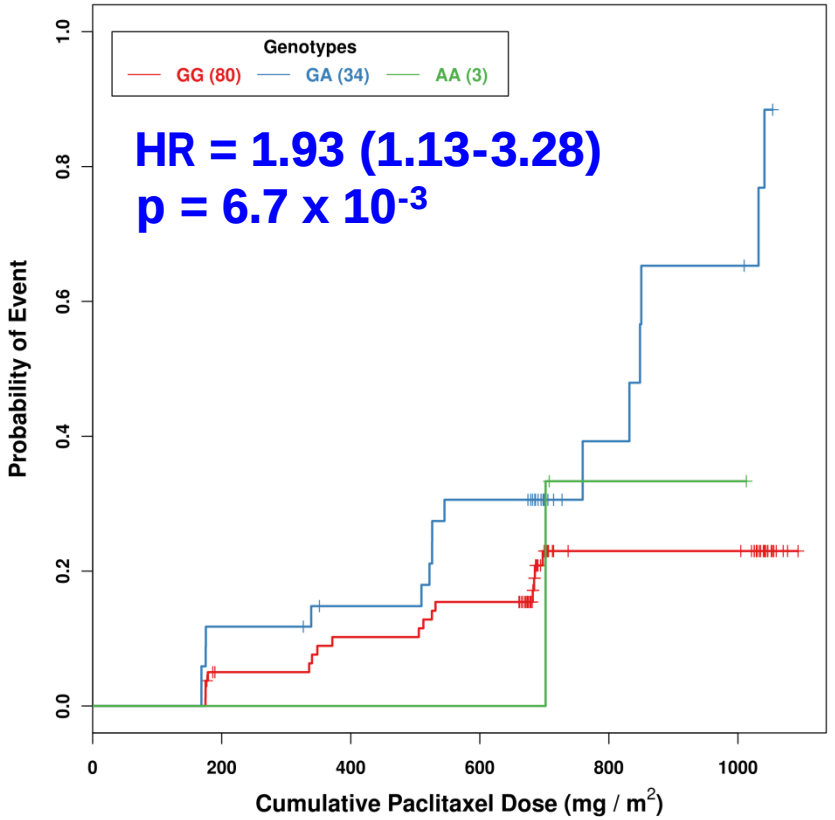
Baldwin et al. *Clin Cancer Res* 18:5099-5109 (2012)

Replication of *FGD4* Association in Europeans and African Americans

Europeans

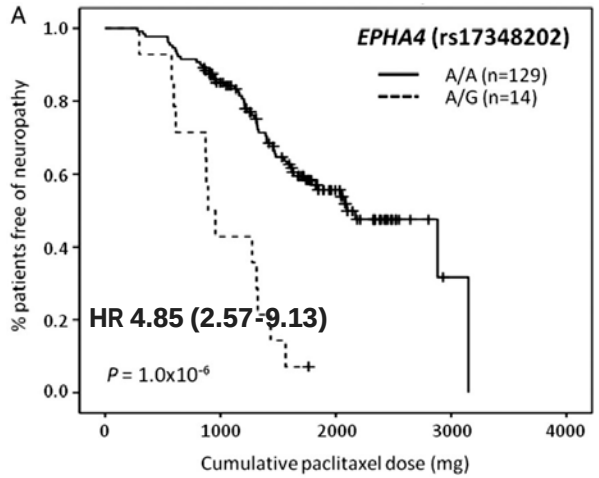


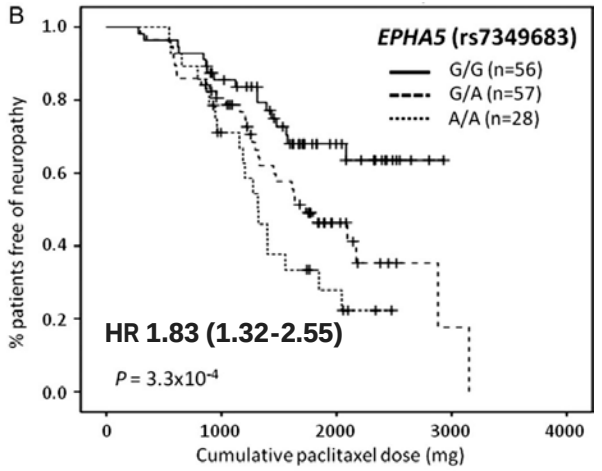
African Americans

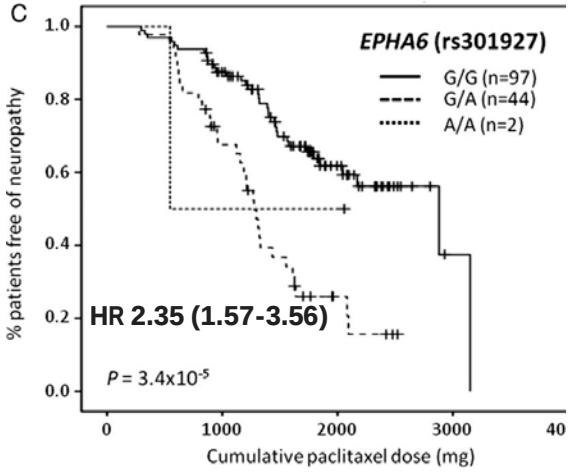


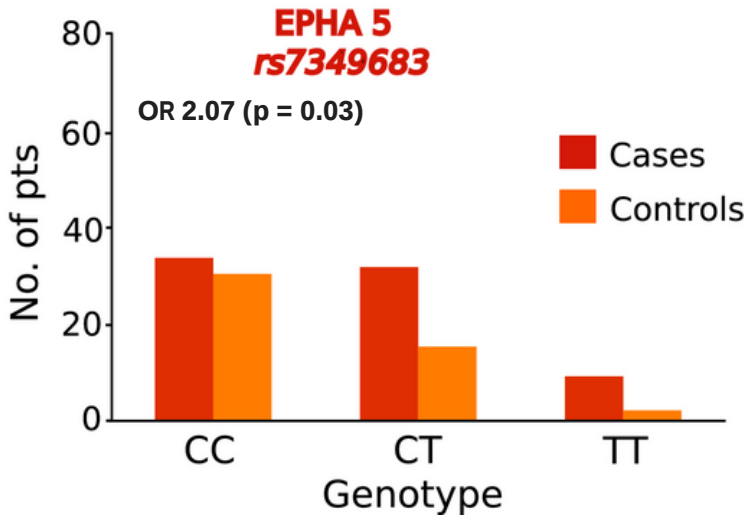
Baldwin et al. *Clin Cancer Res* 18:5099-5109 (2012)

EPHA Receptor Associations Have Been Replicated by Others





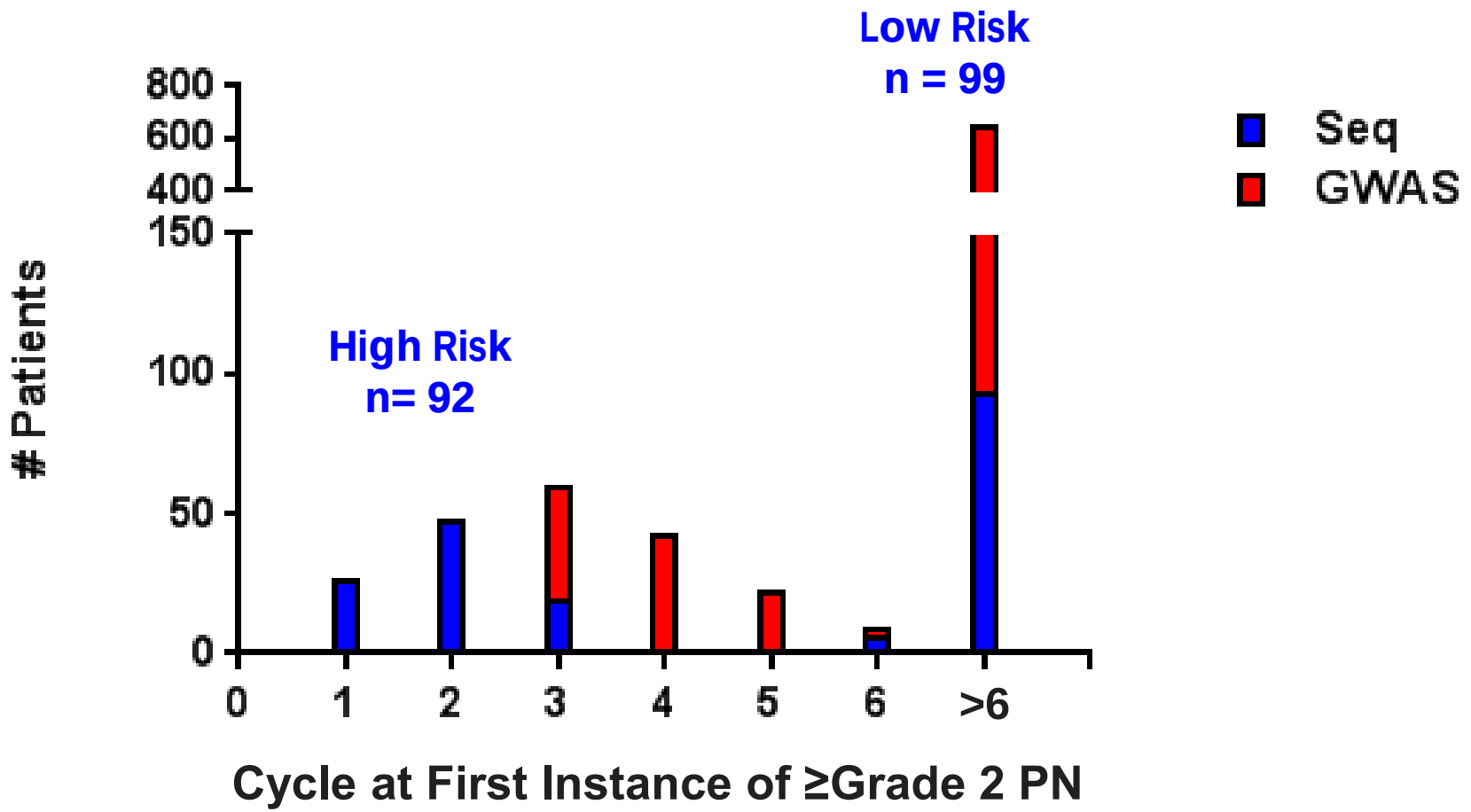




***EPHA6* rs301927**
OR 1.29 (1.07-1.55)
P = 0.008

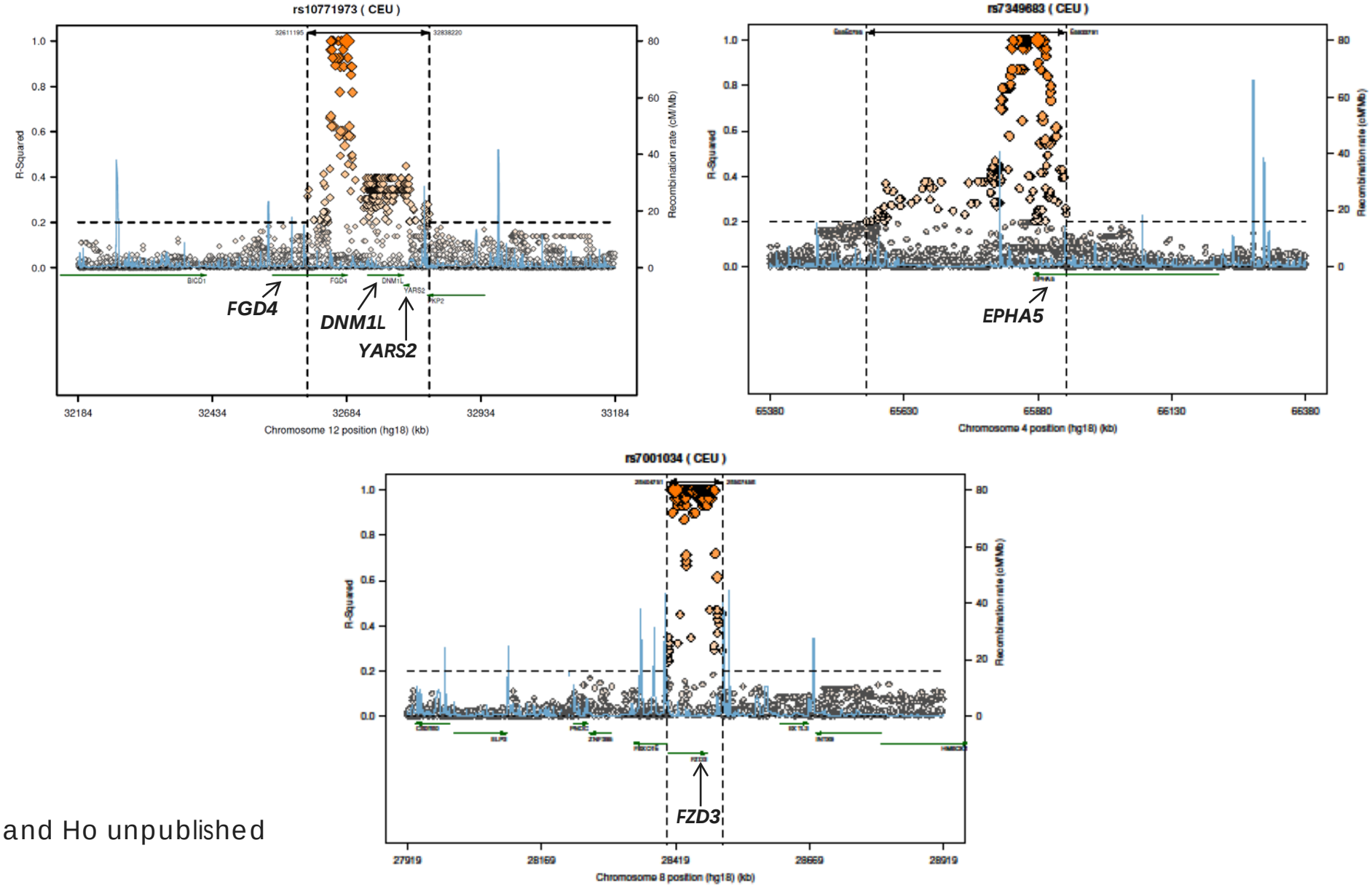
Leandro-Garcia et al. *J Med Genet* 50:599-605 (2013)
Boora et al. *Cancer Med* 5:631-639 (2016)
Abraham et al. *Clin Cancer Res* 20:2466-2475 (2014)

Targeted Resequencing in Tails of CALGB 40101
Neuropathy Distribution



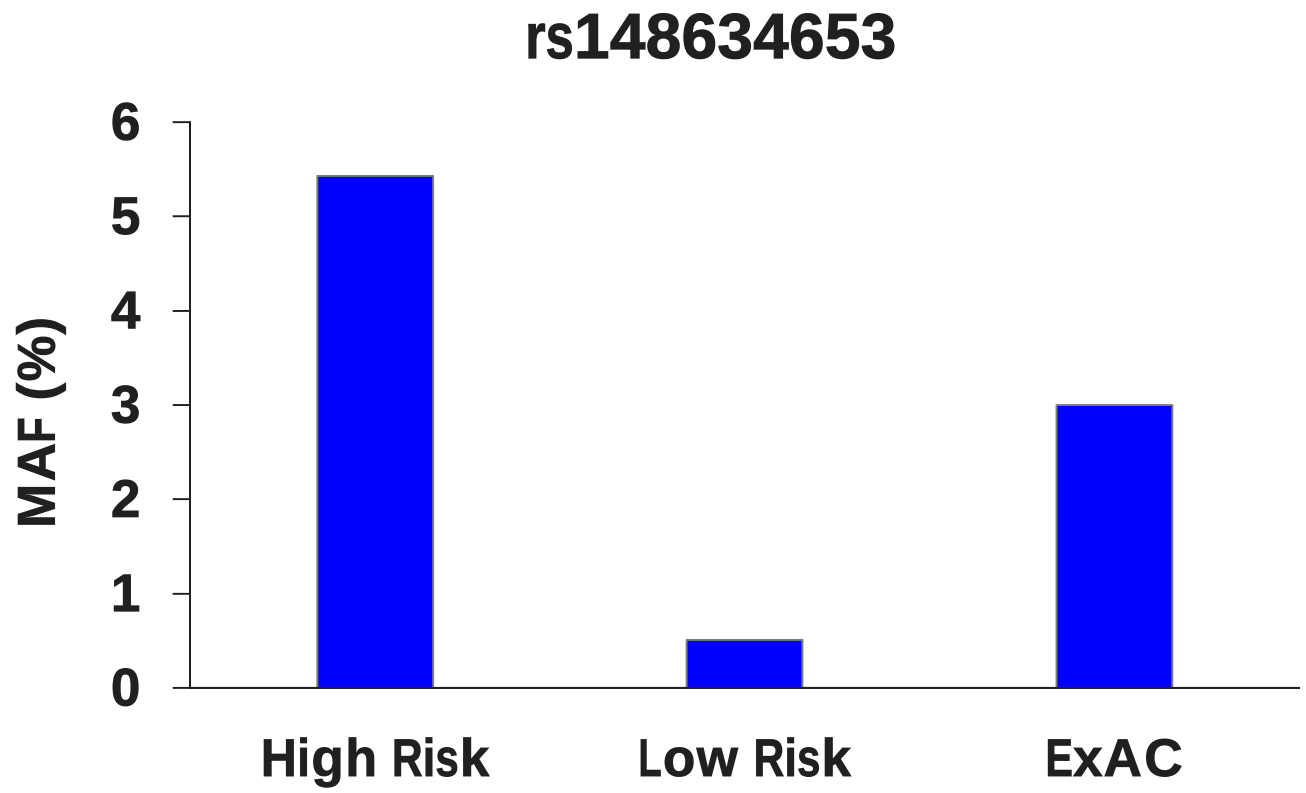
Chhibber, Li and Ho unpublished

Targeted Resequencing Regions



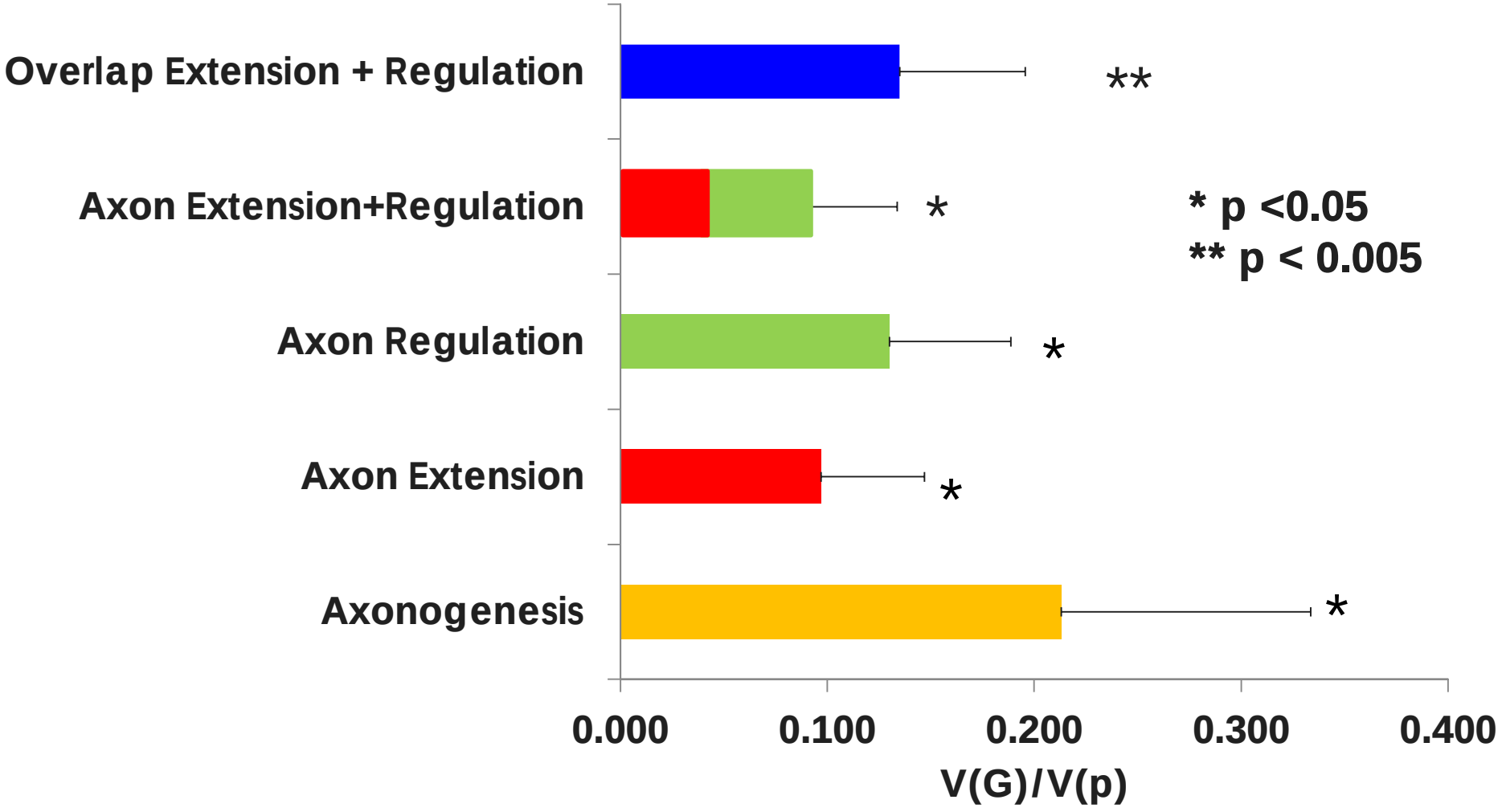
Chhibber, Li and Ho unpublished

DNM1L Synonymous Variant Enriched in High Risk Group



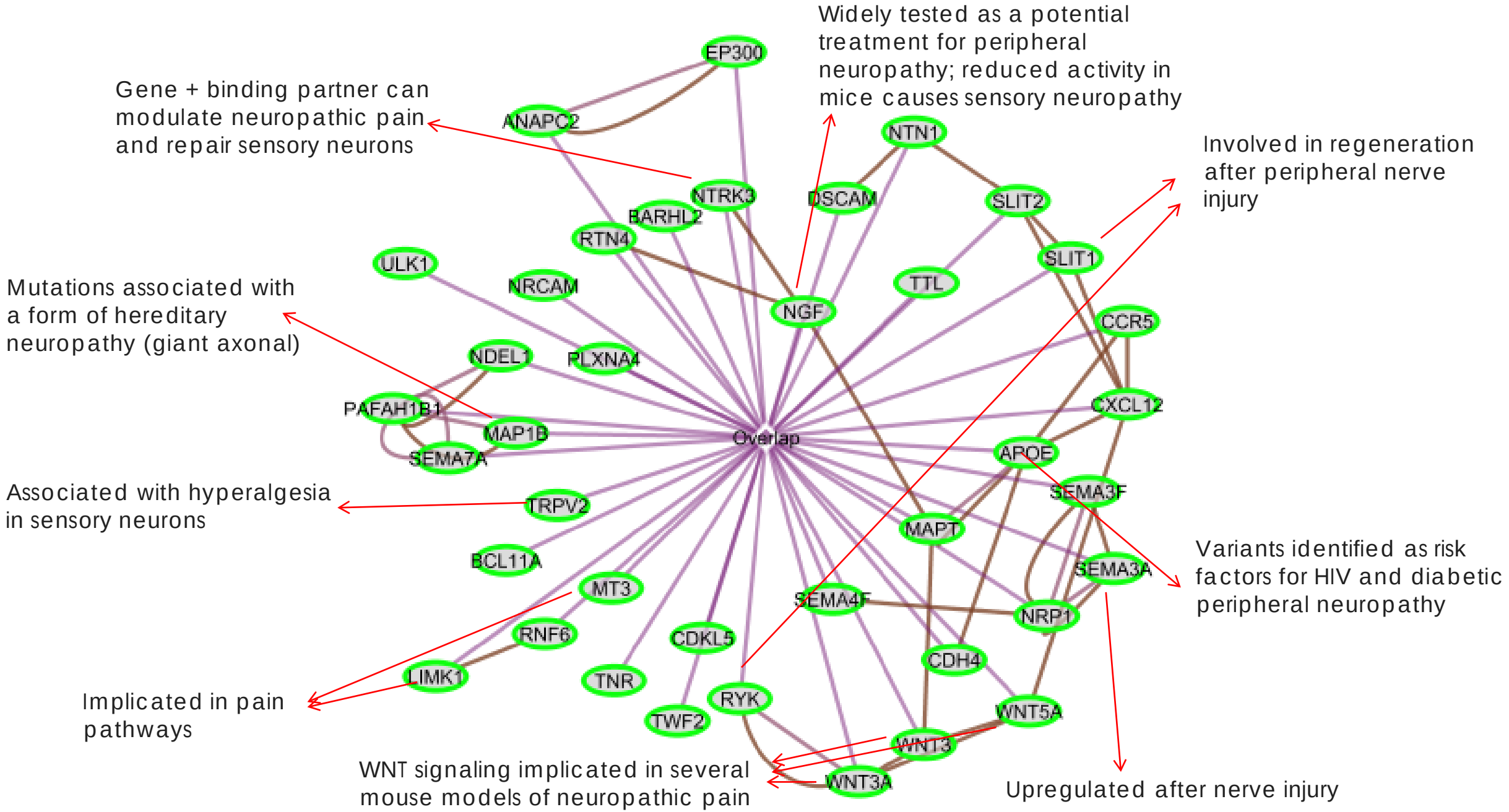
Chhibber, Li and Ho unpublished

Heritability Captured by SNPs in GO Axonogenesis and Axonogenesis Children Sets

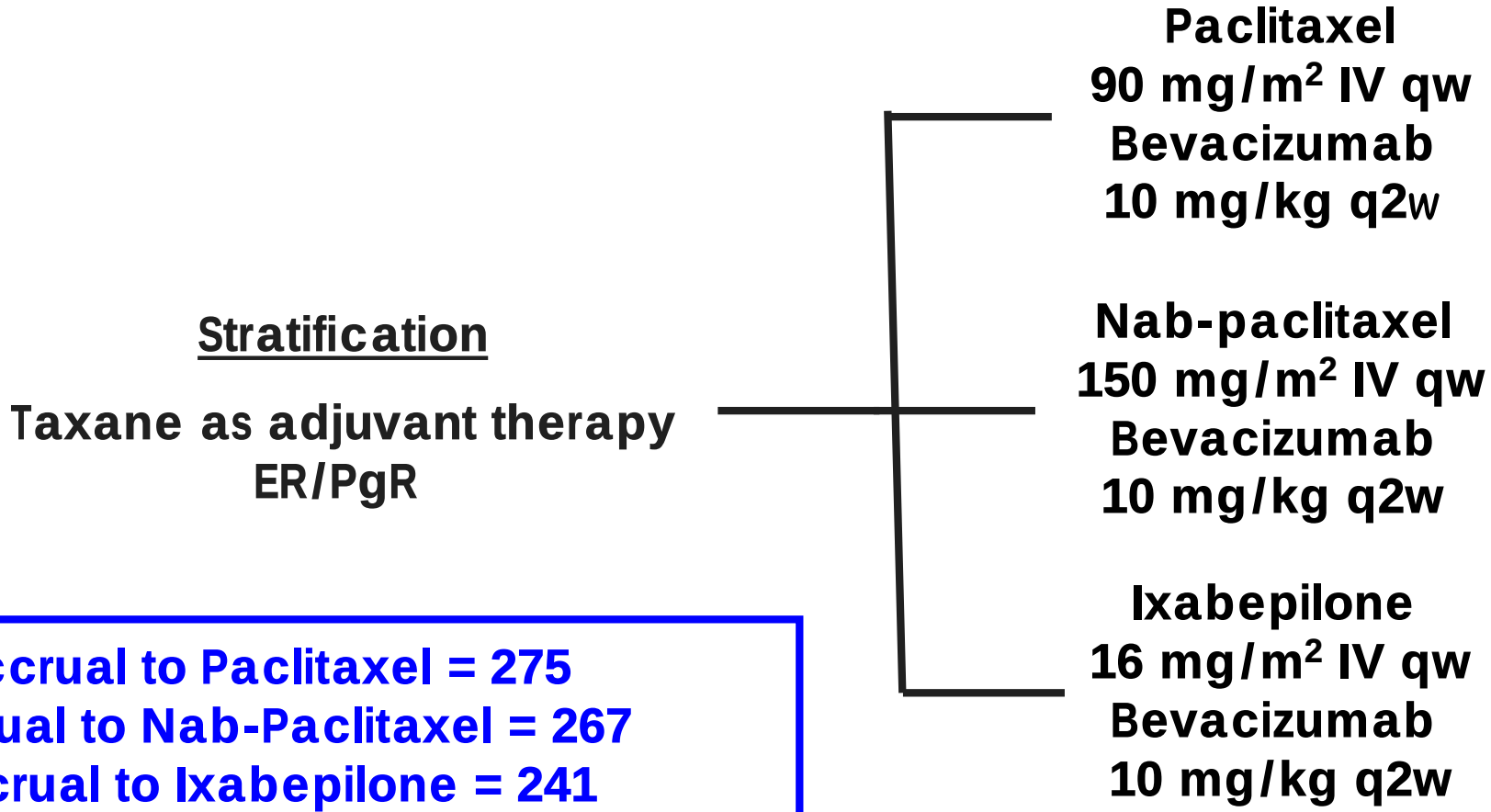


Chhibber et al. *Pharmacogenomics J* 14:336-342 (2014)

Genes in Overlap of Axon Regulation and Extension



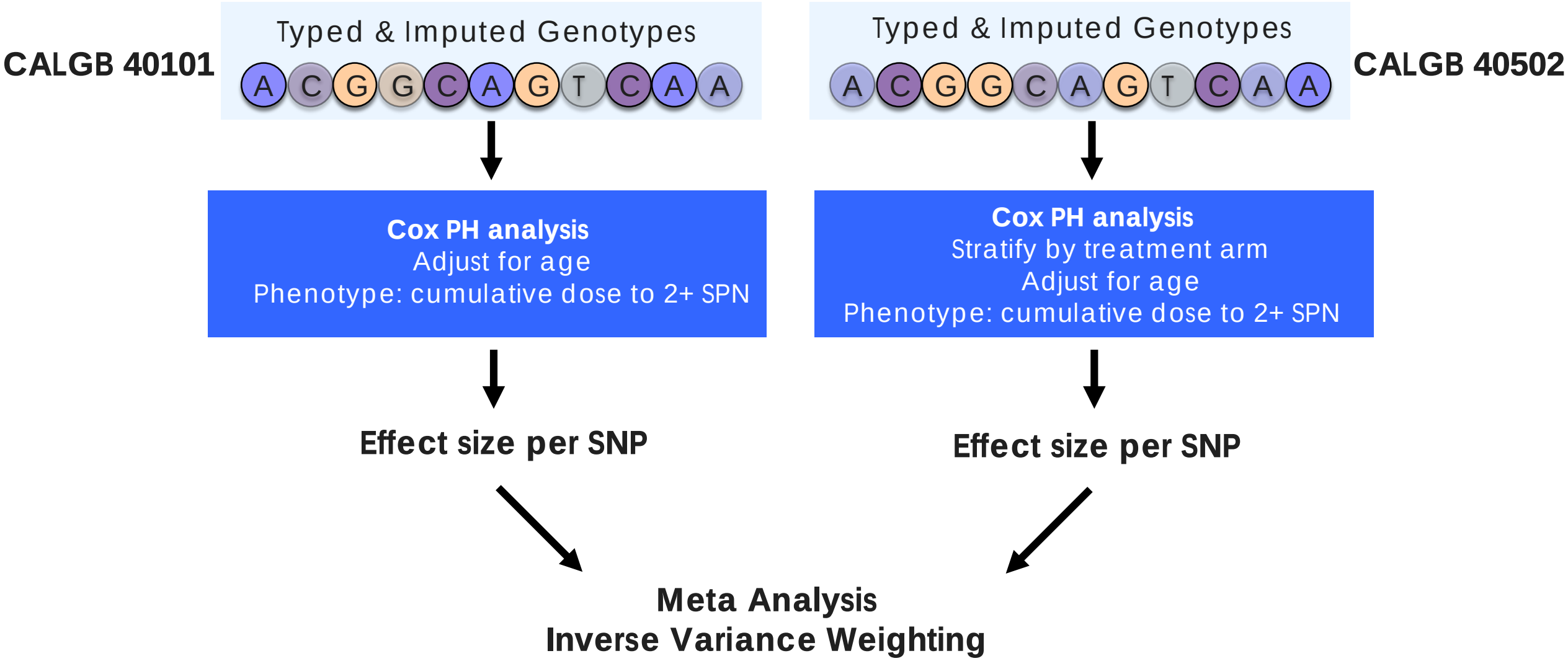
CALGB 40502: Randomized Phase III Trial of Paclitaxel, Nab-Paclitaxel or Ixabepilone with Bevacizumab for Locally Recurrent or Metastatic Breast Cancer



Accrual to Paclitaxel = 275
Accrual to Nab-Paclitaxel = 267
Accrual to Ixabepilone = 241
635 Consented for PG Companion

Rugo et al. *J Clin Oncol 33:2361-2369* (2015)

Meta Analysis of CALGB 40101 and 40502

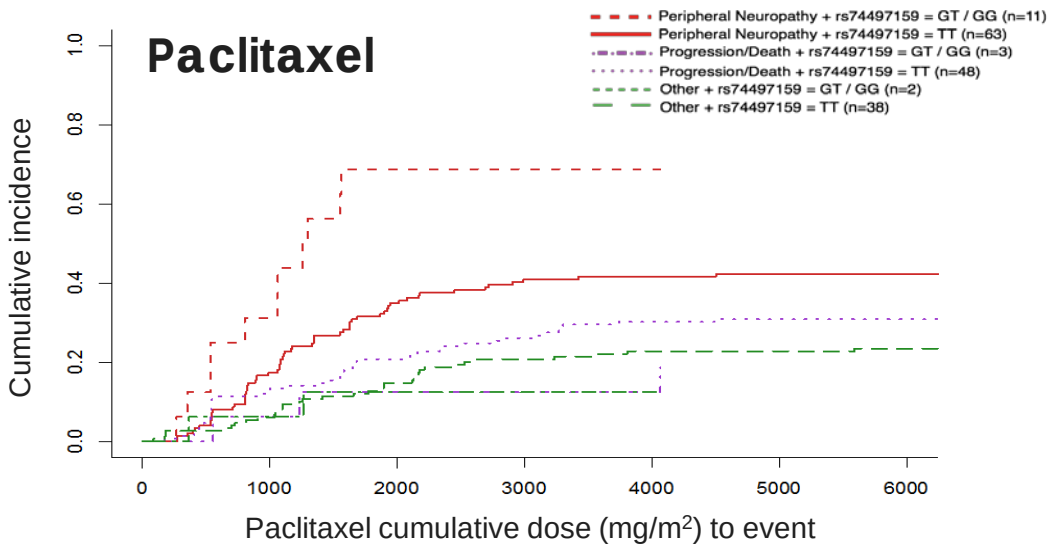


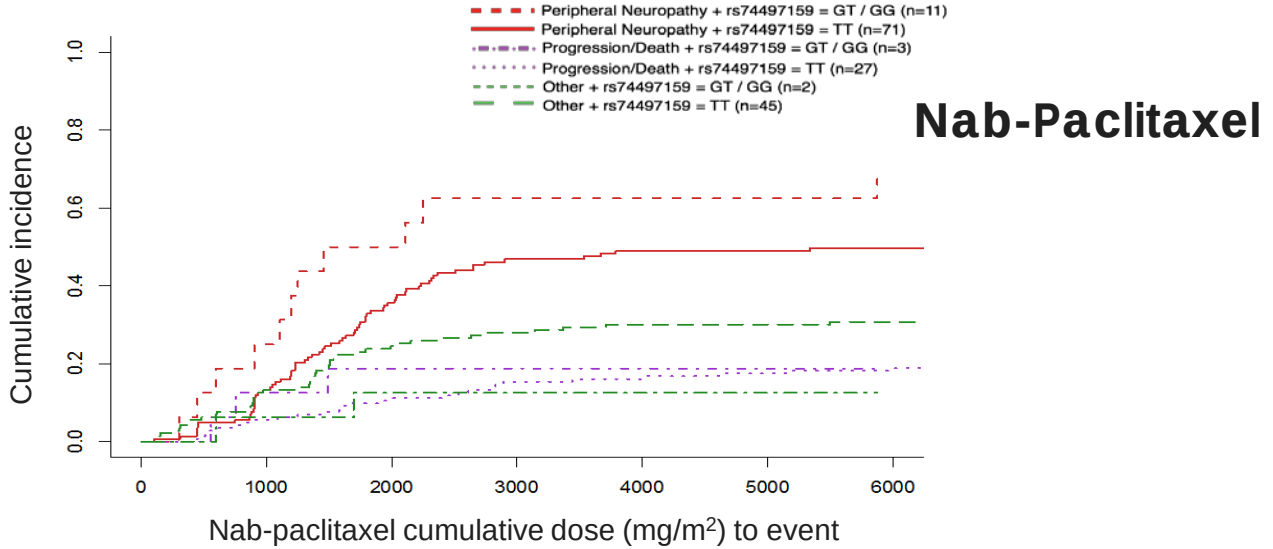
Kat Chua– see poster PI-051

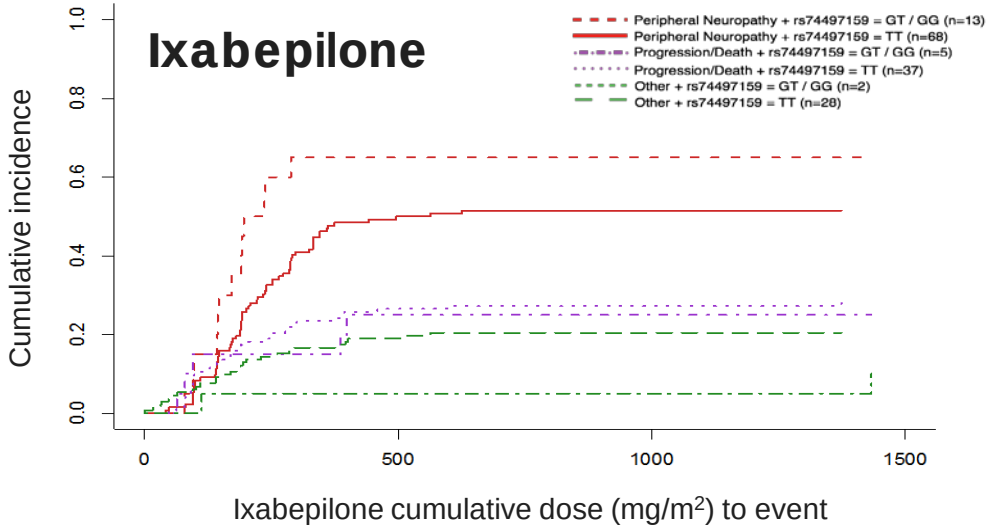
Meta Analysis Identified Novel Genetic Markers of Microtubule Targeting Agent-Induced Peripheral Neuropathy

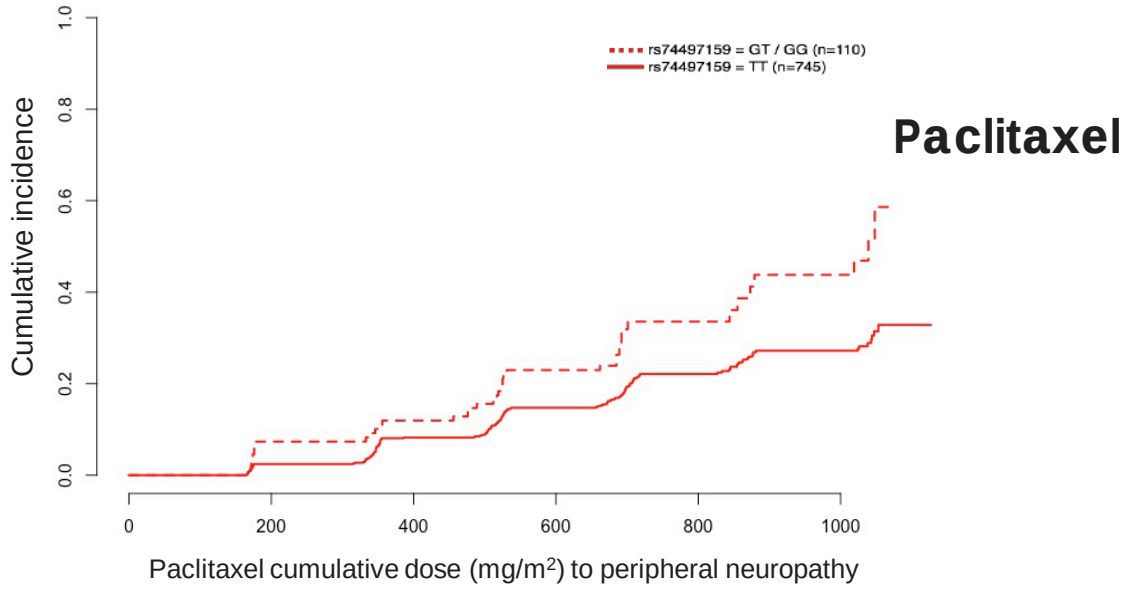
SNP	Gene	CALGB 40101		CALGB 40502		Meta-Analysis		
		HR	*P*	HR	*P*	HR	95% CI	*P*
rs74497159	*S1PR1*	1.81	5.96E-04	2.00	1.59E-04	1.89	1.48-2.43	3.62E-07
rs12738931	*S1PR1*	1.60	4.50E-03	1.88	5.55E-05	1.74	1.39-2.18	1.05E-06
rs79367518	*S1PR1*	1.55	3.58E-03	1.72	1.56E-04	1.63	1.33-2.00	2.06E-06
rs10771973	*FGD4*	1.57	3.91E-06	1.23	3.65E-02	1.39	1.21-1.59	2.15E-06
rs11076190	*CX3CL1*	0.48	3.18E-05	0.64	1.38E-02	0.55	0.43-0.71	2.55E-06
rs9623812	*SCUBE1*	0.71	3.71E-03	0.68	2.60E-04	0.69	0.59-0.81	3.23E-06
rs2060717	*CALU*	1.64	4.35E-03	2.09	1.61E-04	1.83	1.42-2.36	3.48E-06
rs12402160	*S1PR1*	1.44	1.15E-02	1.75	1.22E-04	1.58	1.30-1.93	6.83E-06
rs777619	*BAI3*	1.52	1.54E-04	1.37	1.48E-02	1.45	1.23-1.71	8.13E-06
rs2188156	*SEPT5*	0.45	1.90E-05	0.67	5.28E-02	0.54	0.41-0.71	8.23E-06

SNP Downstream of *S1PR1* is Associated with Microtubule Targeting Agent-Induced Neuropathy









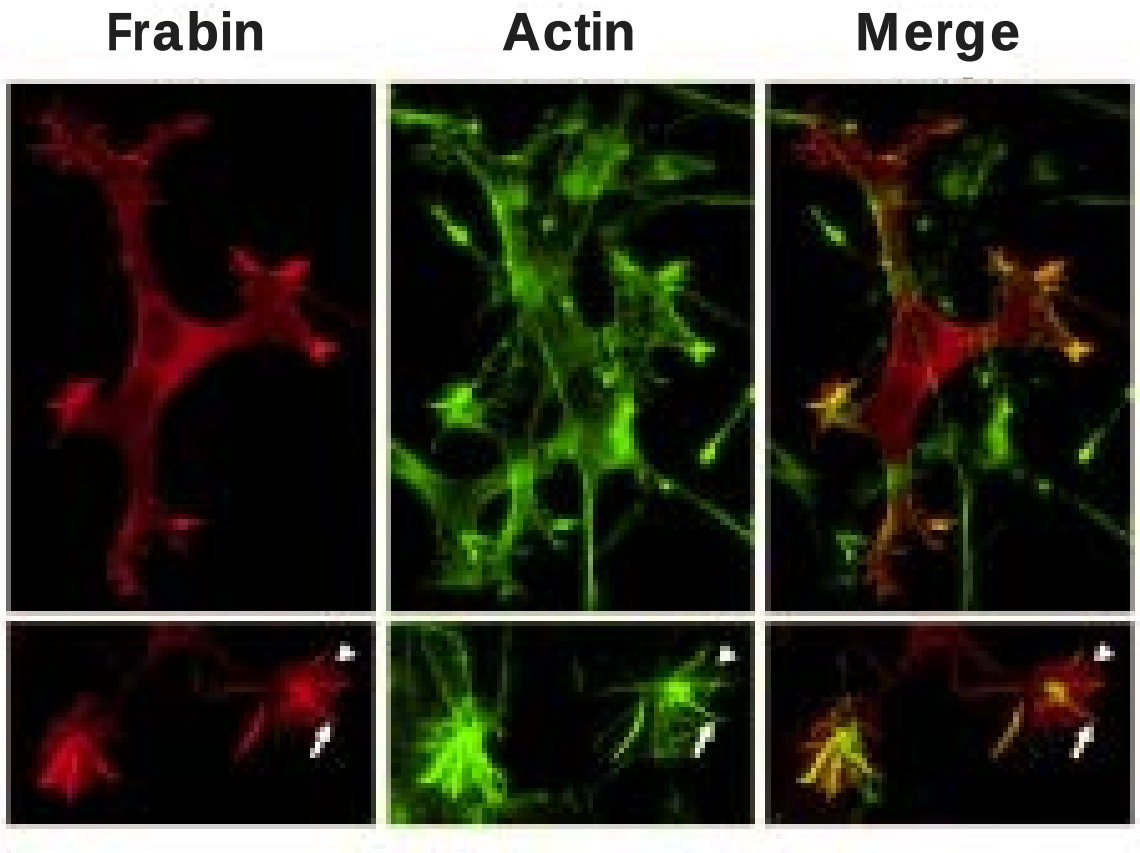
Kat Chua– see poster PI-051

Novel Genes Associated with Paclitaxel-Induced Peripheral Neuropathy

Gene	Function	Replicated?	Biologically Plausible?	CMT Gene?	Expressed in DRG?
FGD4	RhoGEF for Cdc42, cell shape	√	√	√	√
EPHA4/5/6/8	Receptor tyrosine kinase, axon guidance	√	√		√
ARHGEF10	RhoGEF, slow nerve conduction velocity	√	√	√	√
S1PR1	Sphingosine signaling, neuroinflammation		√		√
DNM1L	Mitochondrial fission, GTPase		√		√
FZD3	Wnt signaling, axon guidance		√		√
PRX	Myelin maintenance		√	√	√
TRPV1	Pain receptor		√		√
SBF2	GEF for RAB28		√	√	√
FCAMR	Immune function				

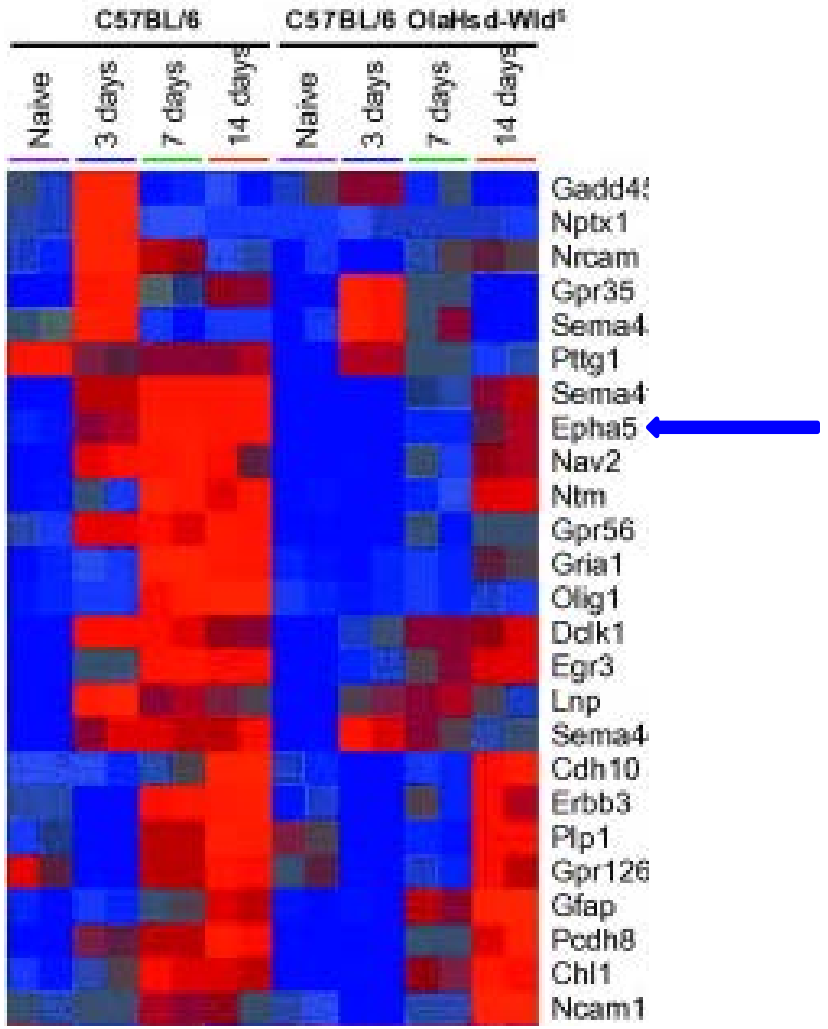
Cliff et al. *Crit Rev Oncol Hematol* 120: 127 (2017)

Frabin/FGD4 Induces Cdc42-mediated Filopidia/Lamellipodia Formation



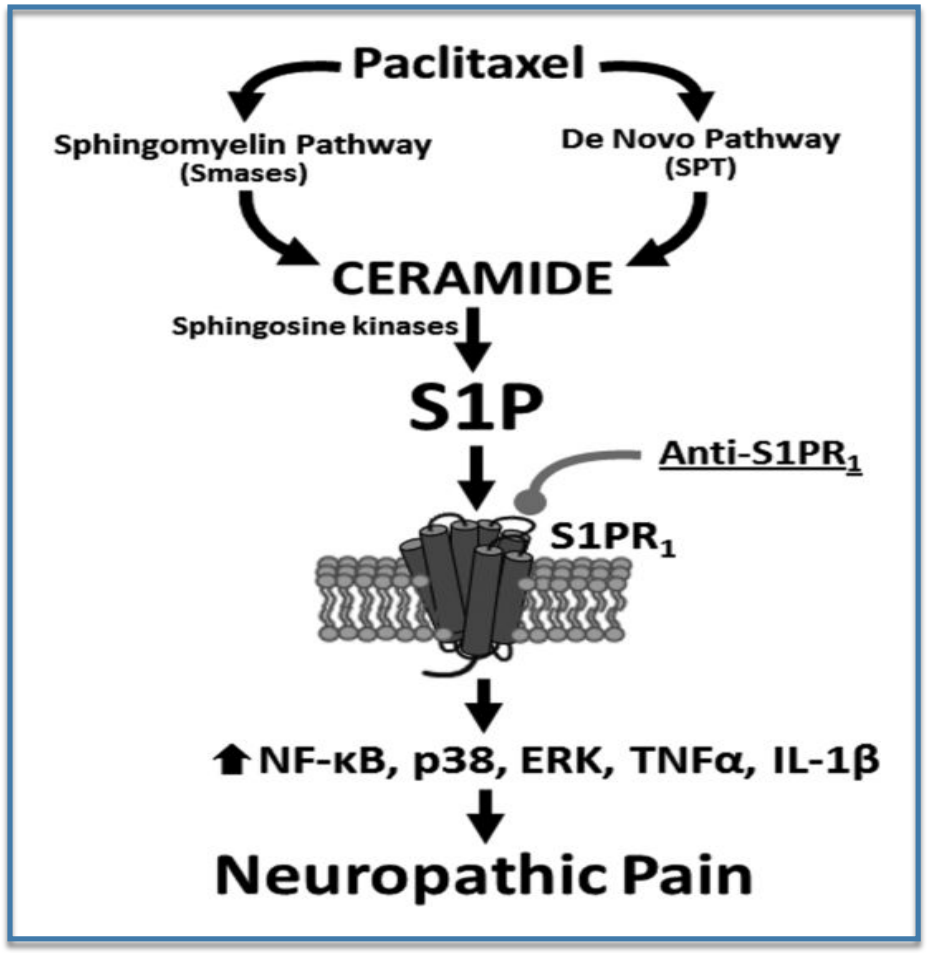
Stendel et al. *Am J Hum Genet* 81:158-164 (2007)

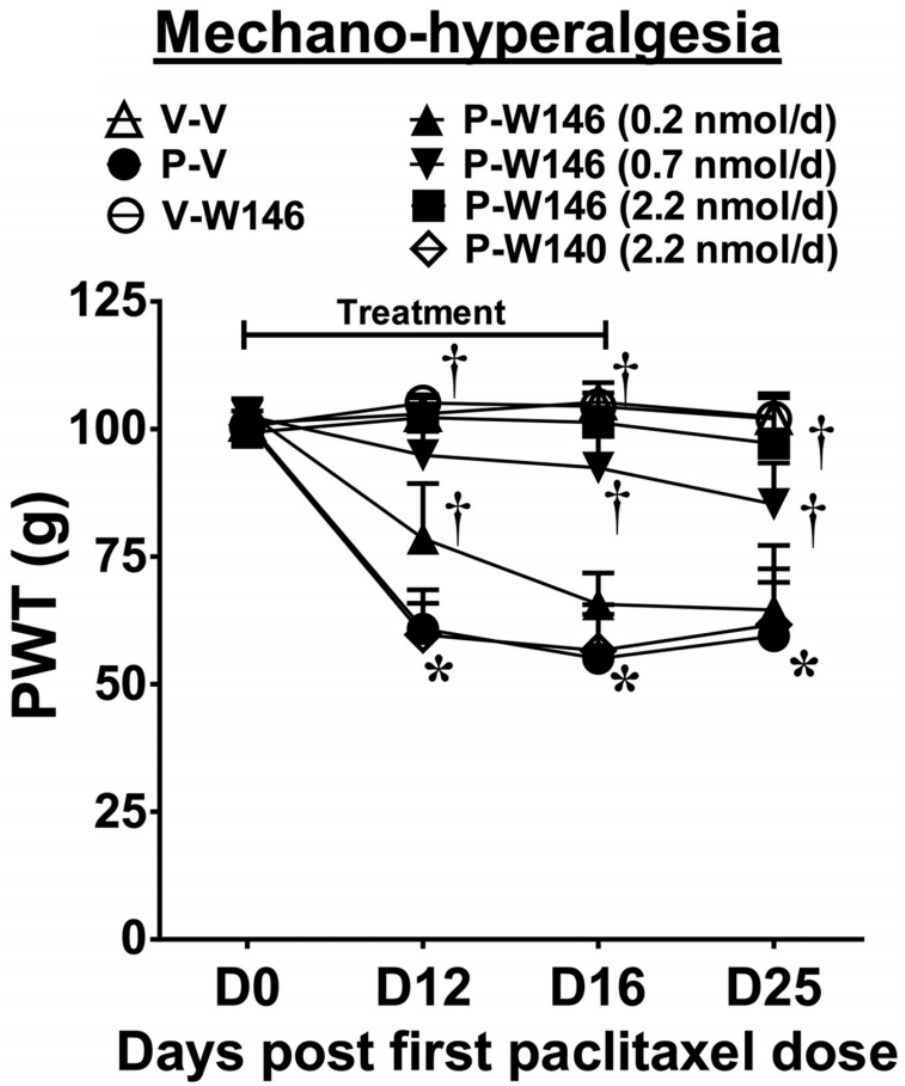
EphrinA5 Expression is Attenuated in a Mouse Model of Peripheral Nerve Injury



Barrette et al. *Brain, Behavior and Immunity* 24:1254-1267 (2010)

Inhibition of S1PR1 Attenuates Paclitaxel-Induced Neuropathic Pain

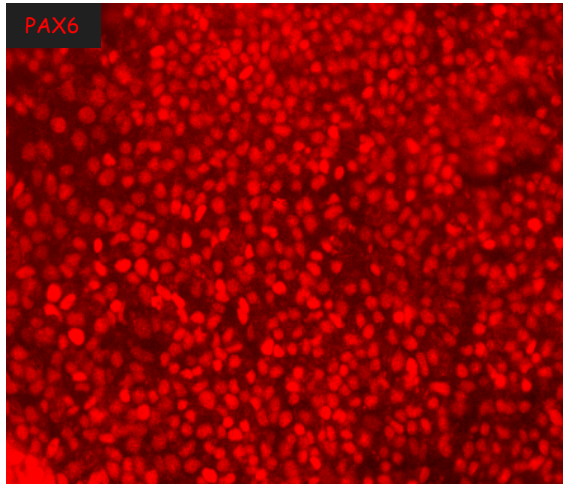




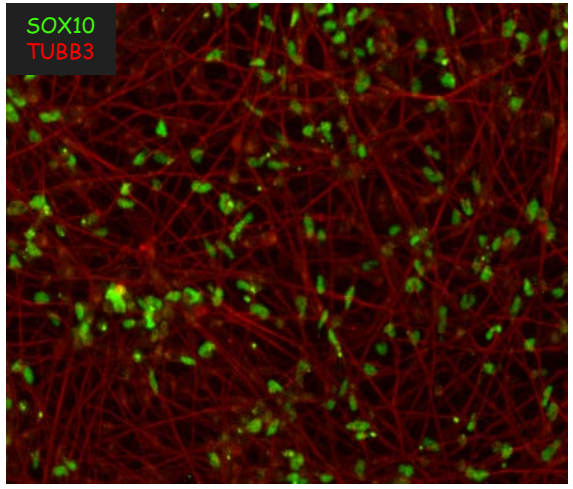
Janes et al *J Biol Chem* 289:21082-21097 (2014)

Human iPSC-Derived Sensory Neurons

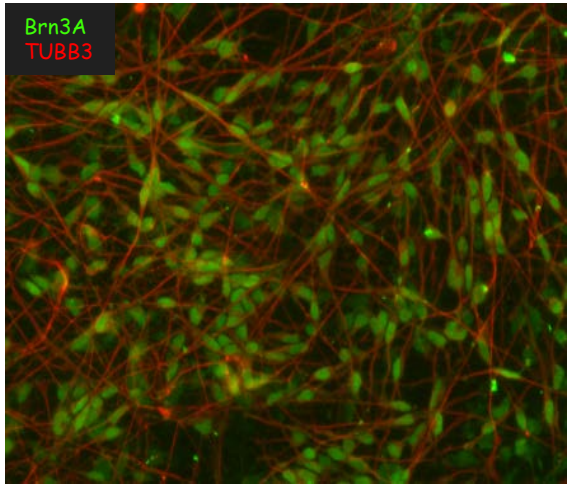
Neurocrest
PAX6 (day 4)



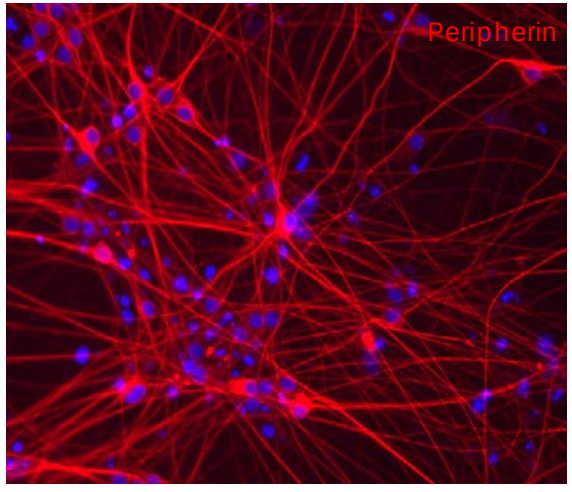
Neuroectoderm
SOX10/TUBB3 (day 12)



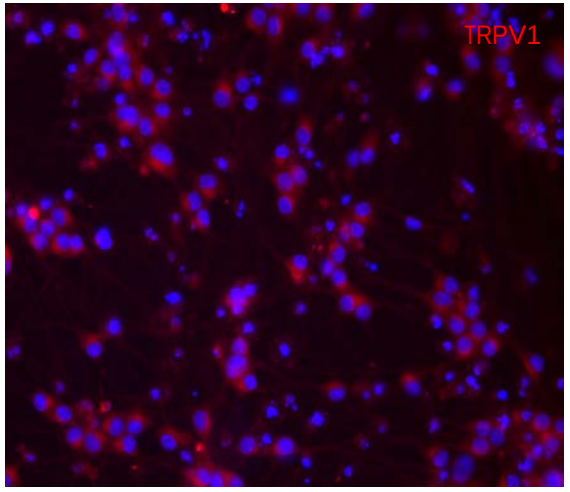
Sensory Neuron
BRN3A/TUBB3 (day 12)



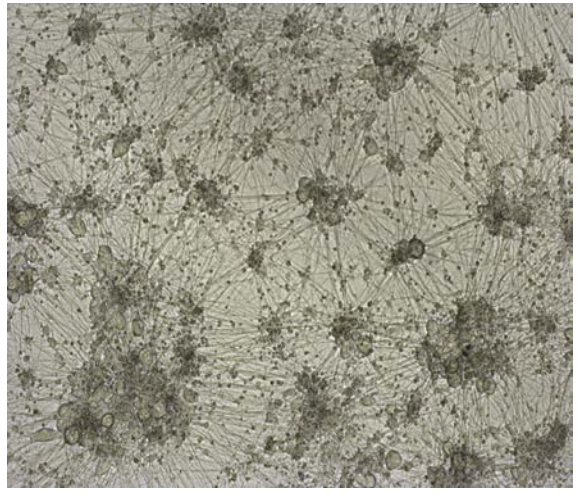
Sensory Neuron
Peripherin (day 24)



Sensory Neuron
TRPV1 (day 25)

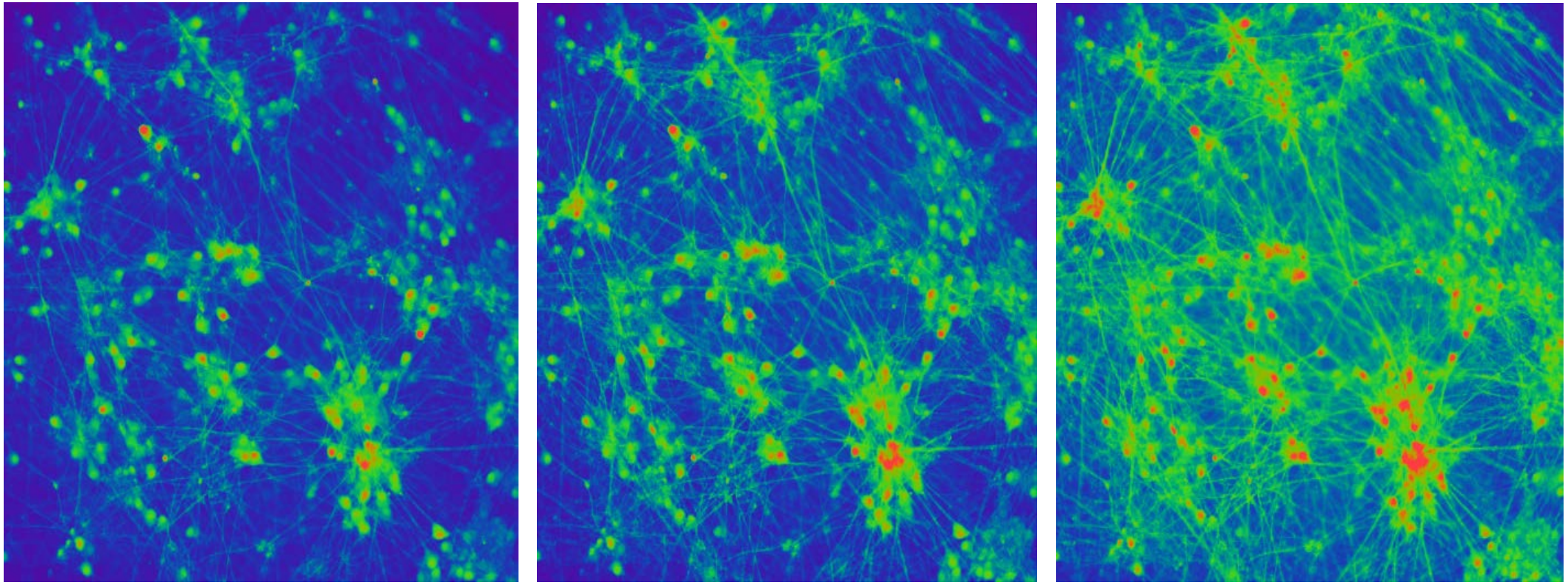


Sensory Neuron
(day 50)



Tore Stage
Chenling Xiong
Annie Altman

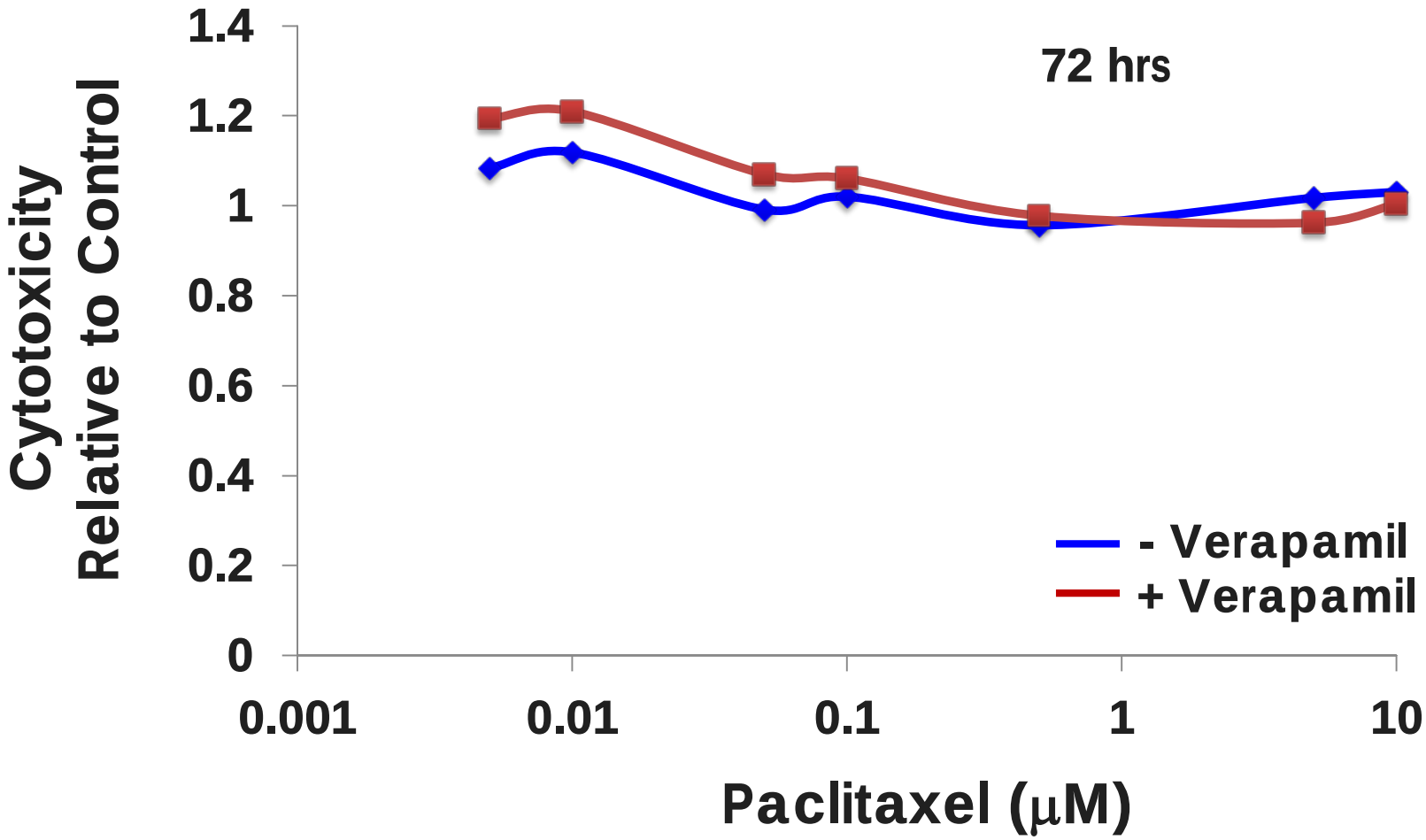
Human iPSC-Derived Sensory Neurons Have Expected Channel and Receptor Activity



Control | **1 μM Capsaicin** | **25 mM KCl**

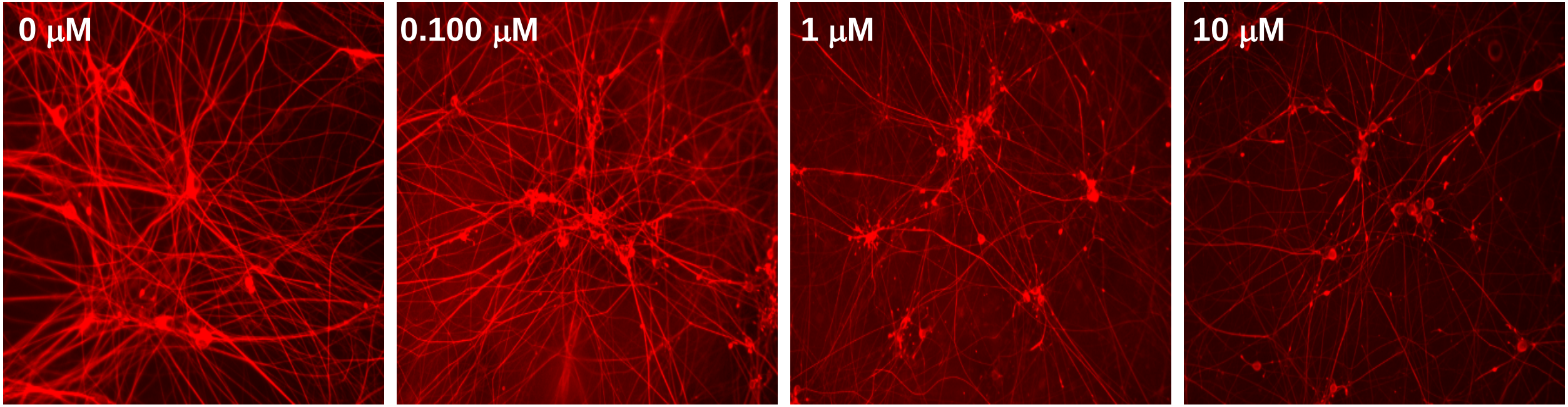
Tore Stage
Chenling Xiong
Annie Altman

Human iPSC-Derived Sensory Neurons are Resistant to Paclitaxel



Tore Stage
Chenling Xiong
Annie Altman

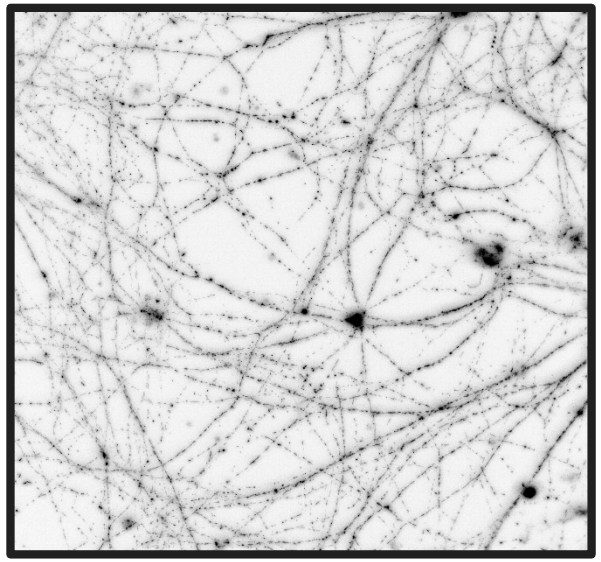
Paclitaxel Affects Neurite Networks in iPSC-Derived Sensory Neurons

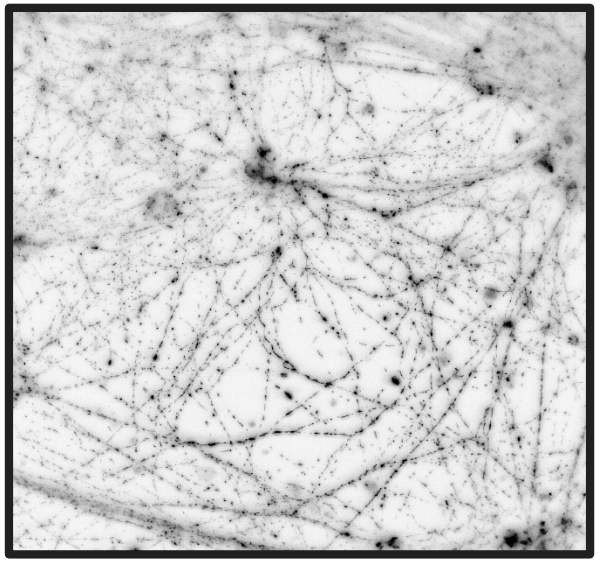


Tore Stage
Chenling Xiong
Annie Altman

Paclitaxel Causes Mitochondrial Aggregation in Human iPSC-Derived Sensory Neurons

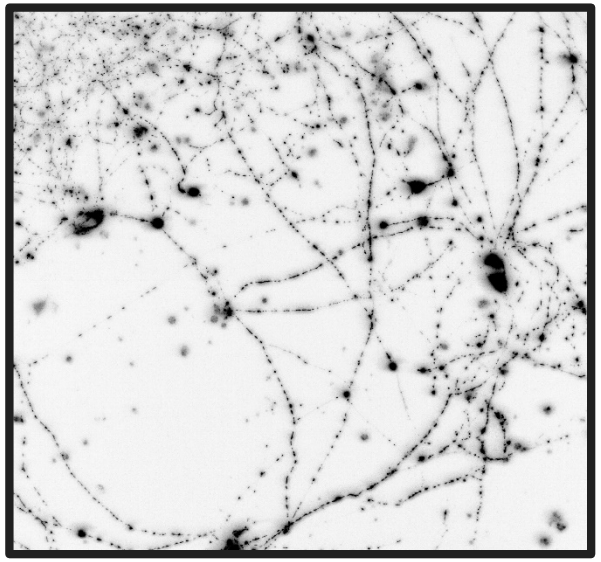
Control

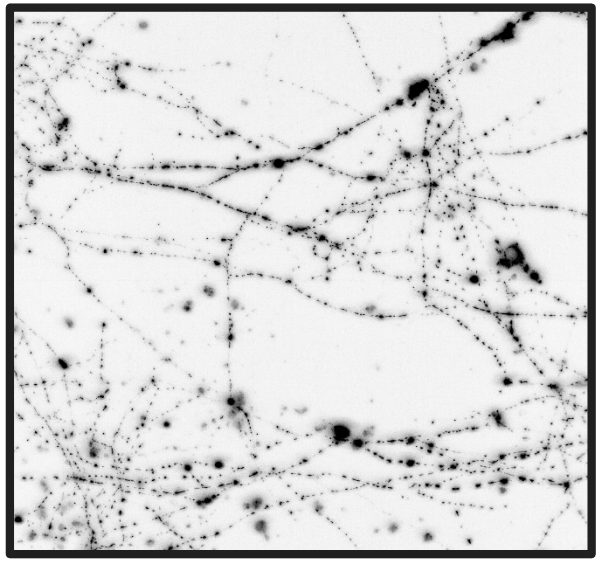




Paclitaxel 10 μM
24 hr

Paclitaxel 10 μM
48 hr

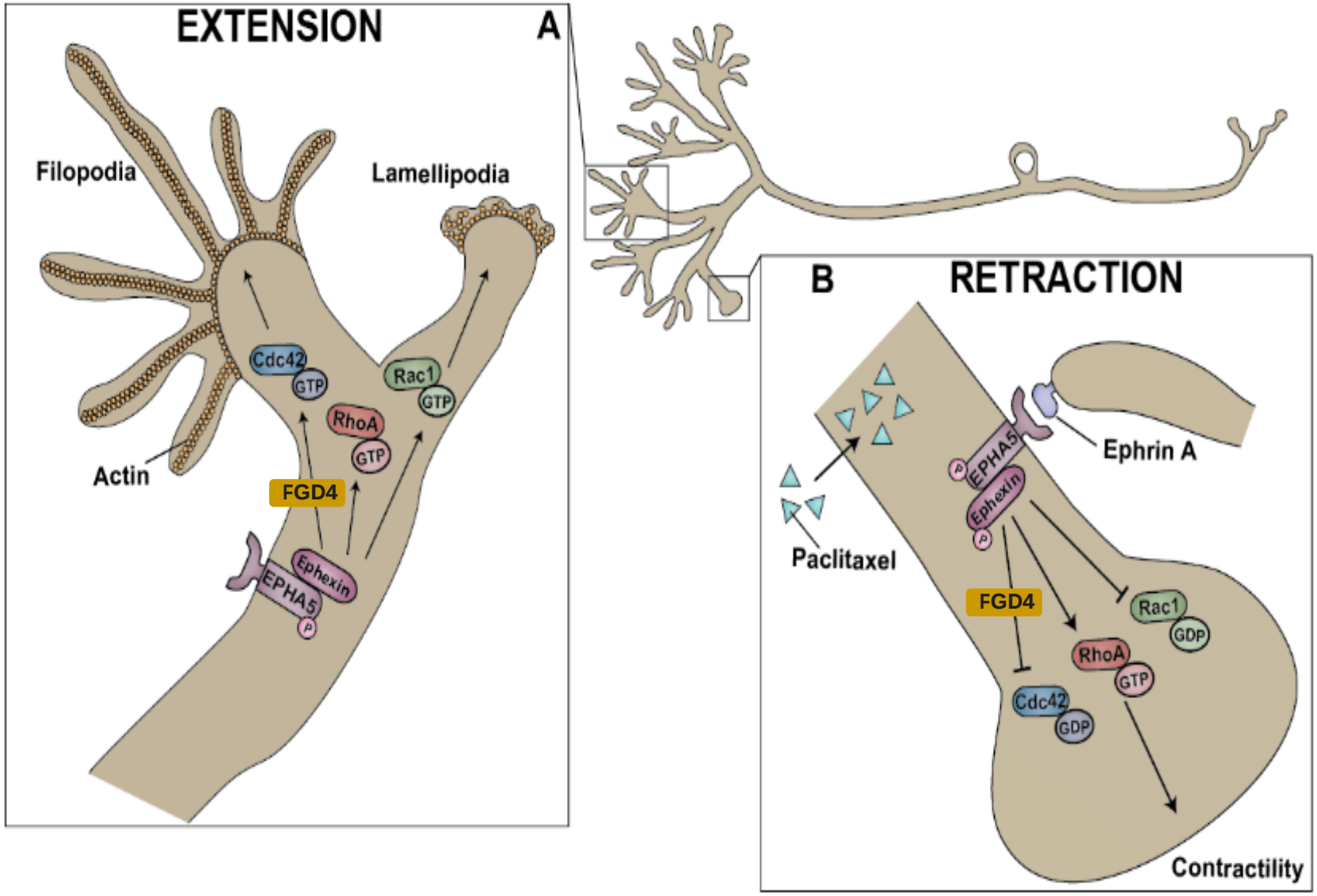




Paclitaxel 10 μM
72 hr

Tore Stage
Chenling Xiong
Annie Altman

Hypothesis: Paclitaxel-Induced EPHA5 and FGD4 Signaling Modulates Rho/Rac/Cdc42 GTPase Activity and Actinomysin Contractility



Conclusions

- Genome-wide studies identified variants in several genes involved in neuron function that are associated with microtubule targeting agent-induced sensory peripheral neuropathy
- Genes involved in axonogenesis contribute significantly to the heritability of this adverse event
- Human iPSC-derived sensory neurons are a robust model for understanding the molecular basis of this toxicity

Current Focus

Replication and discovery of new risk variants and genes

- Exome sequencing of 622 samples from CALGB 40502 to discover new risk variants and genes - Including regulatory regions of candidate genes
- Replication in BioVU and other cohorts
- Meta-analysis with other NCI cooperative groups

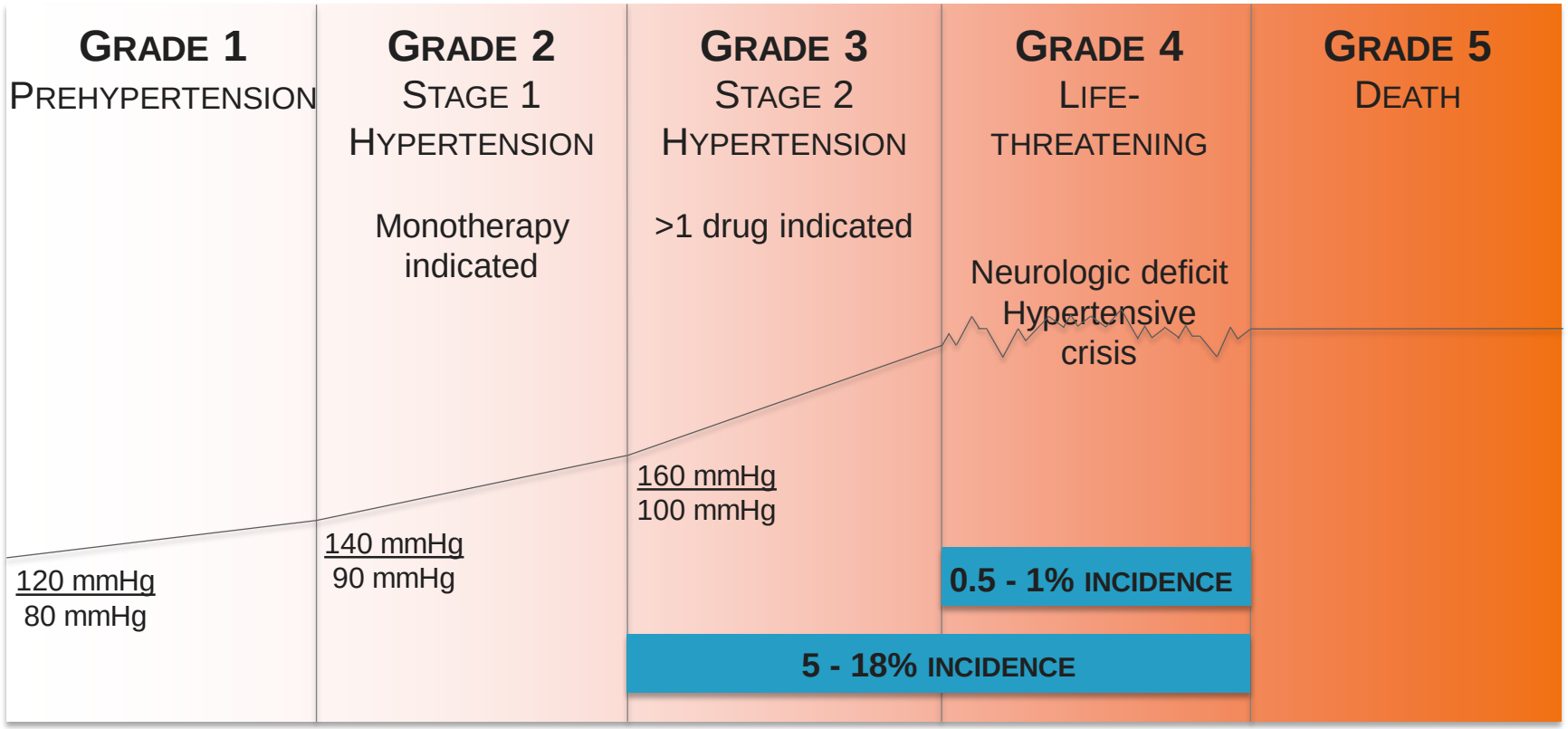
Mechanistic studies of genetic findings

- CRISPR being used to understand gene function and to identify causal variants
- FDA library screen underway
- iPSC-derived Schwann cells
- Mouse studies

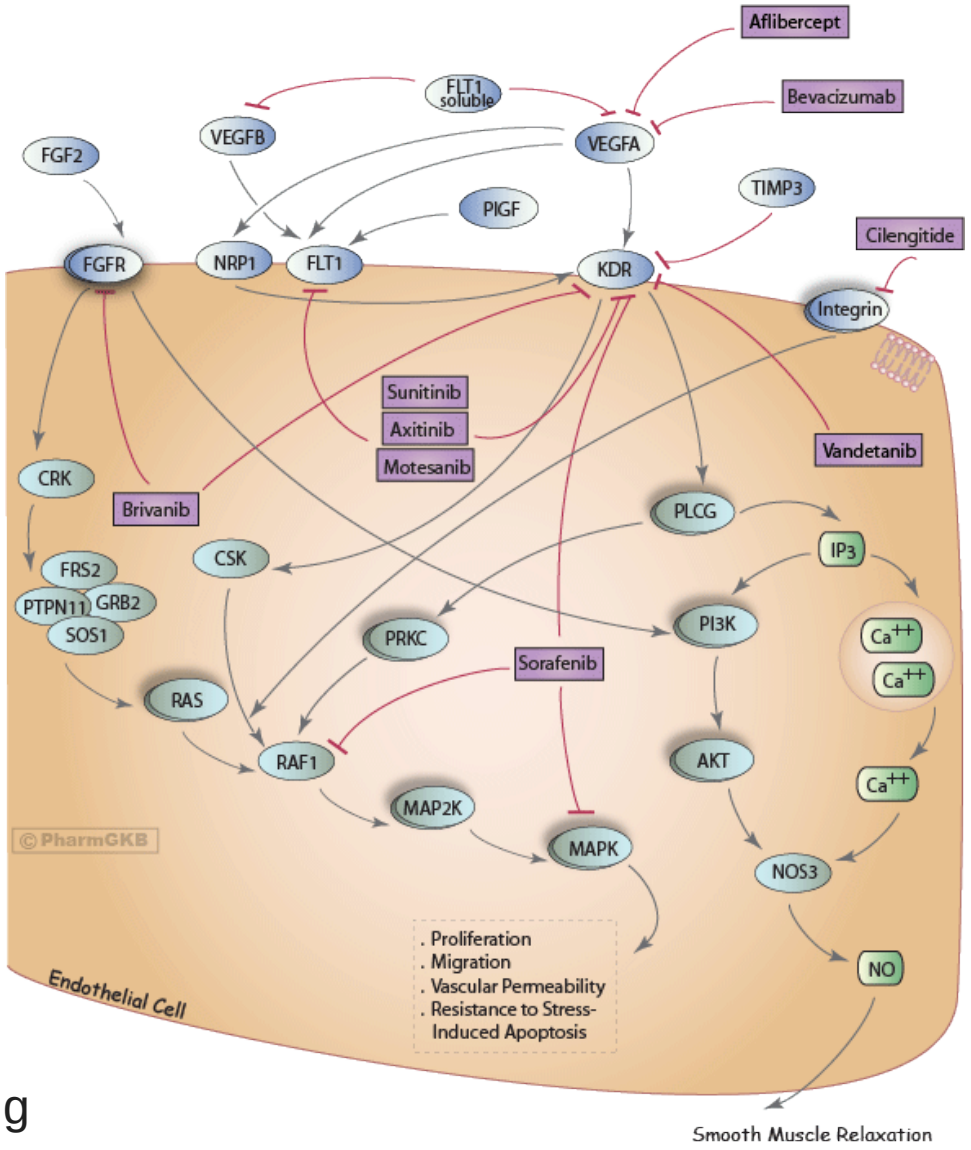
Two Tales of Reverse Translation: From Genomics towards Mechanism

- Chemotherapy-induced peripheral neuropathy
  - GWAS
  - iPSC-Induced sensory neuron studies
- **Bevacizumab-induced hypertension**
  - **Exome sequencing**
  - **Cell-based studies**

Bevacizumab-Induced Hypertension

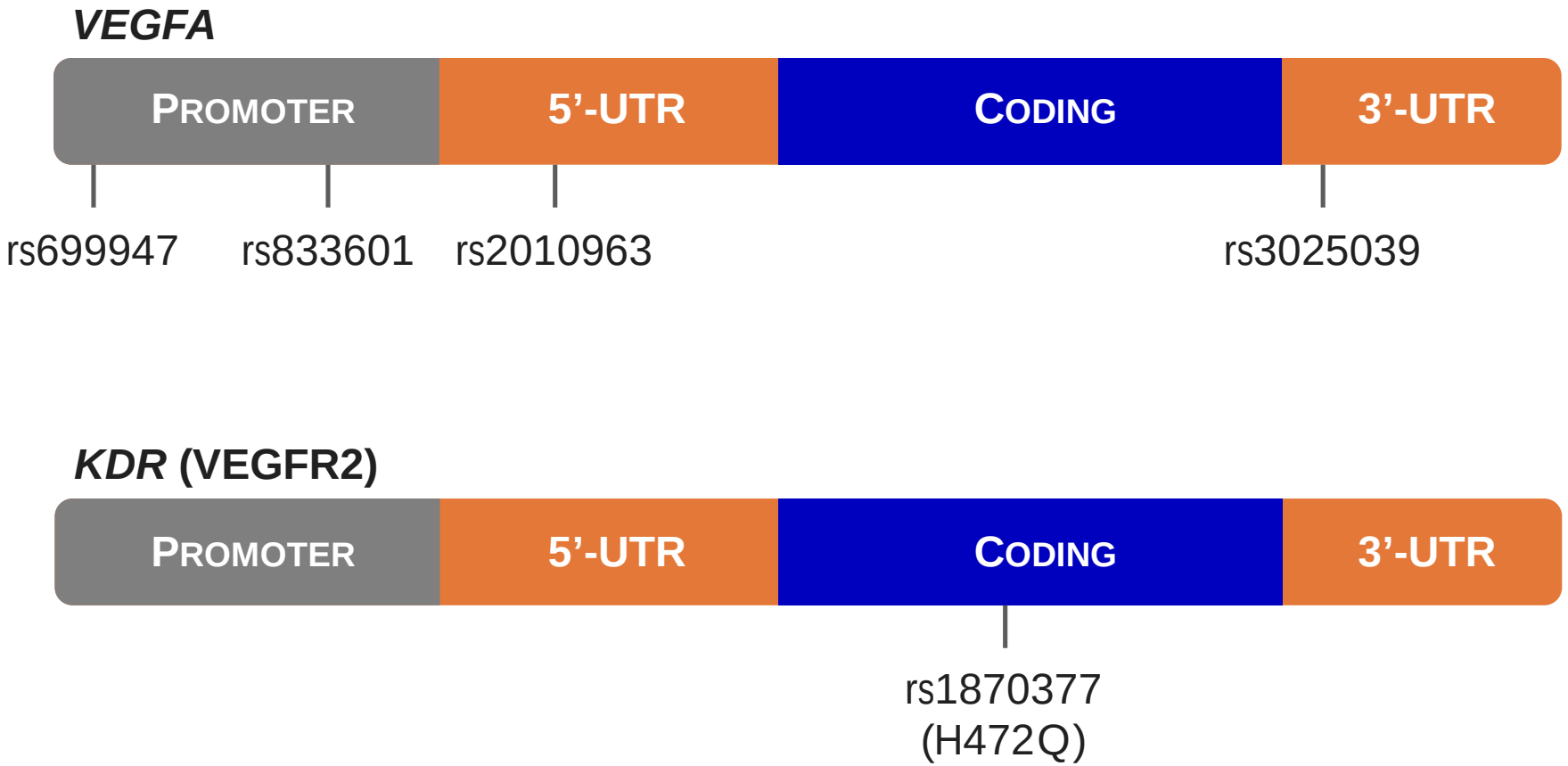


Inhibition of VEGF Signaling Predicted to Disrupt Vascular Tone



www.pharmgkb.org

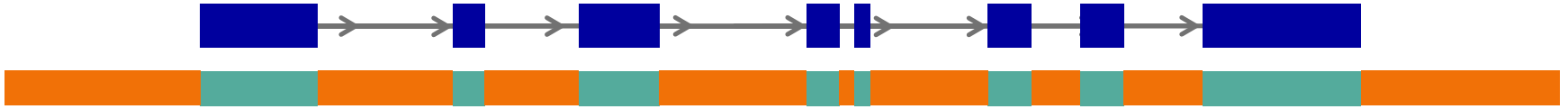
Candidate Gene Studies Identified VEGFA/VEGFR2 Variants Associated With Bevacizumab-Induced Hypertension



Morita et al. *Cancer Chemother Pharmacol* 71:405-411 (2013)
Schneider et al. *J Clin Oncol* 26:4672-4678 (2008)
Etienne-Grimaldi et al *Br J Clin Pharmacol* 71:921-928 (2011)
Jain et al *J Exp Clin Cancer Res* 29:95 (2010)

No validation

Exome Sequencing of Extreme Phenotypes



All genes: Exonic regions (64 Mb)

+

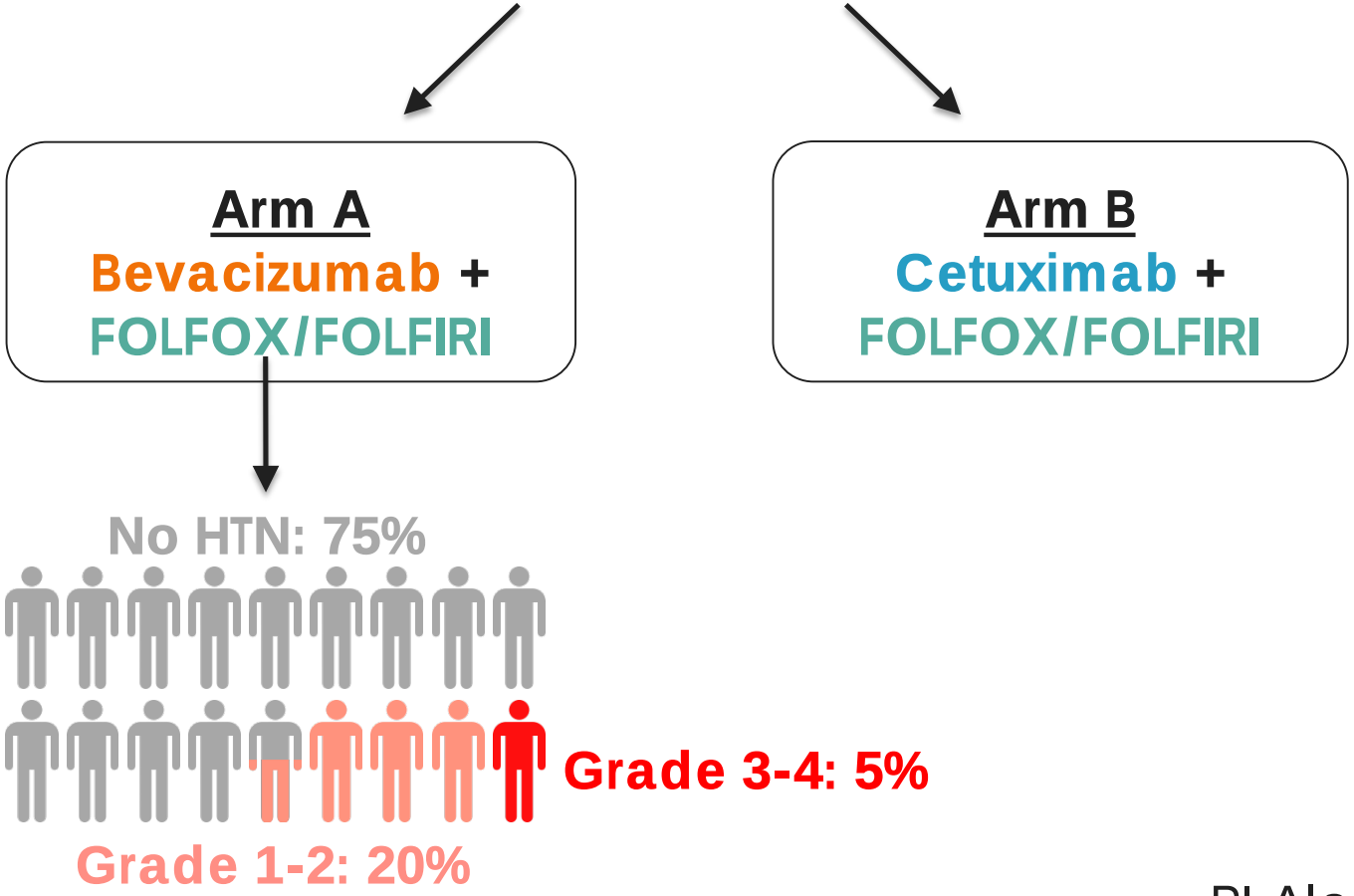
181 Candidate genes: UTRs, introns, ±50 kb upstream/ downstream (22 Mb)

VEGF signaling
Nitric oxide signaling
Hypertension
Endothelial cell biology
Published association hits

Megan Li *Clin Cancer Res* in press

CALGB 80405

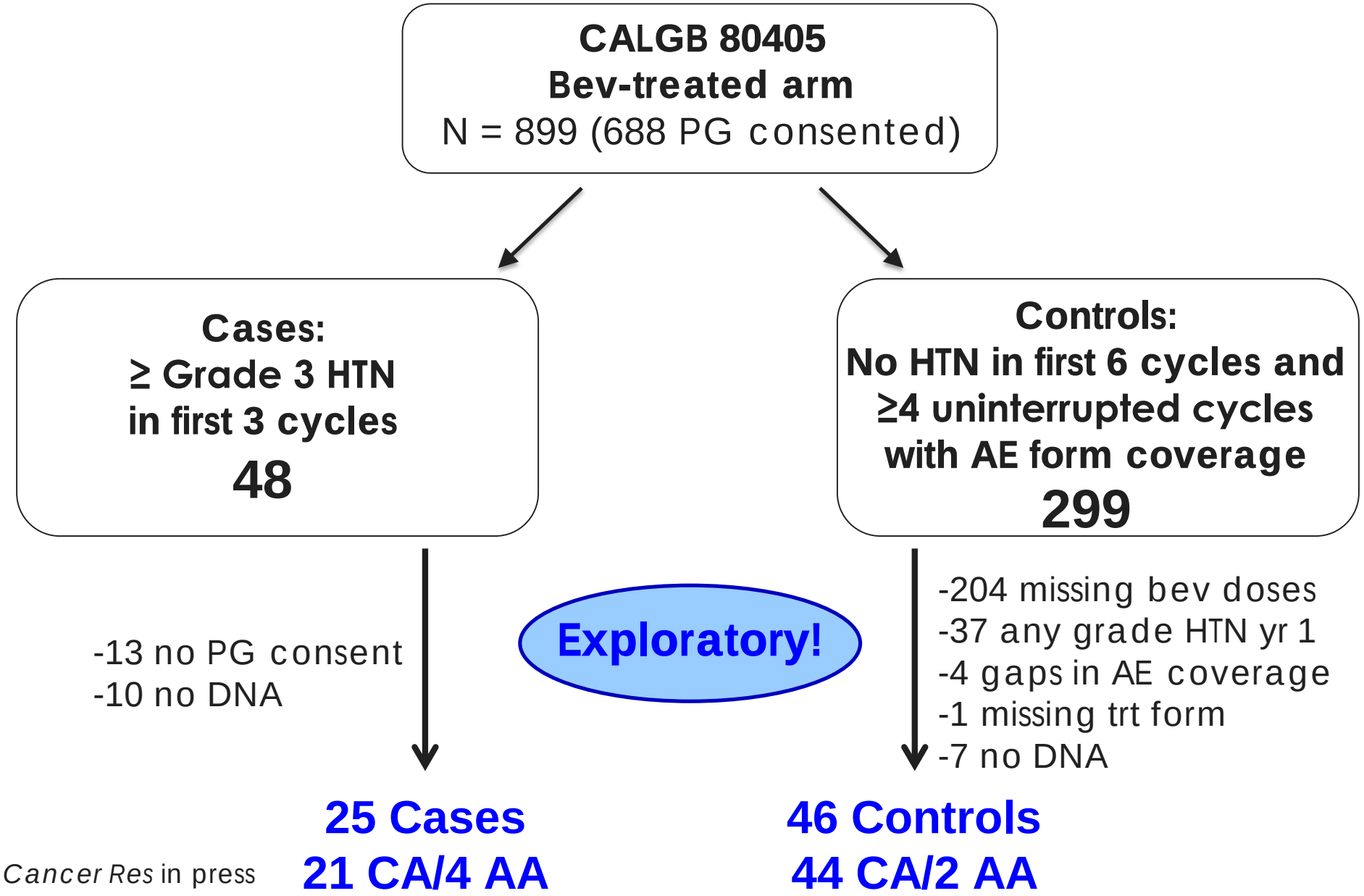
PHASE III: COLORECTAL CANCER TREATMENT



Megan Li *Clin Cancer Res* in press

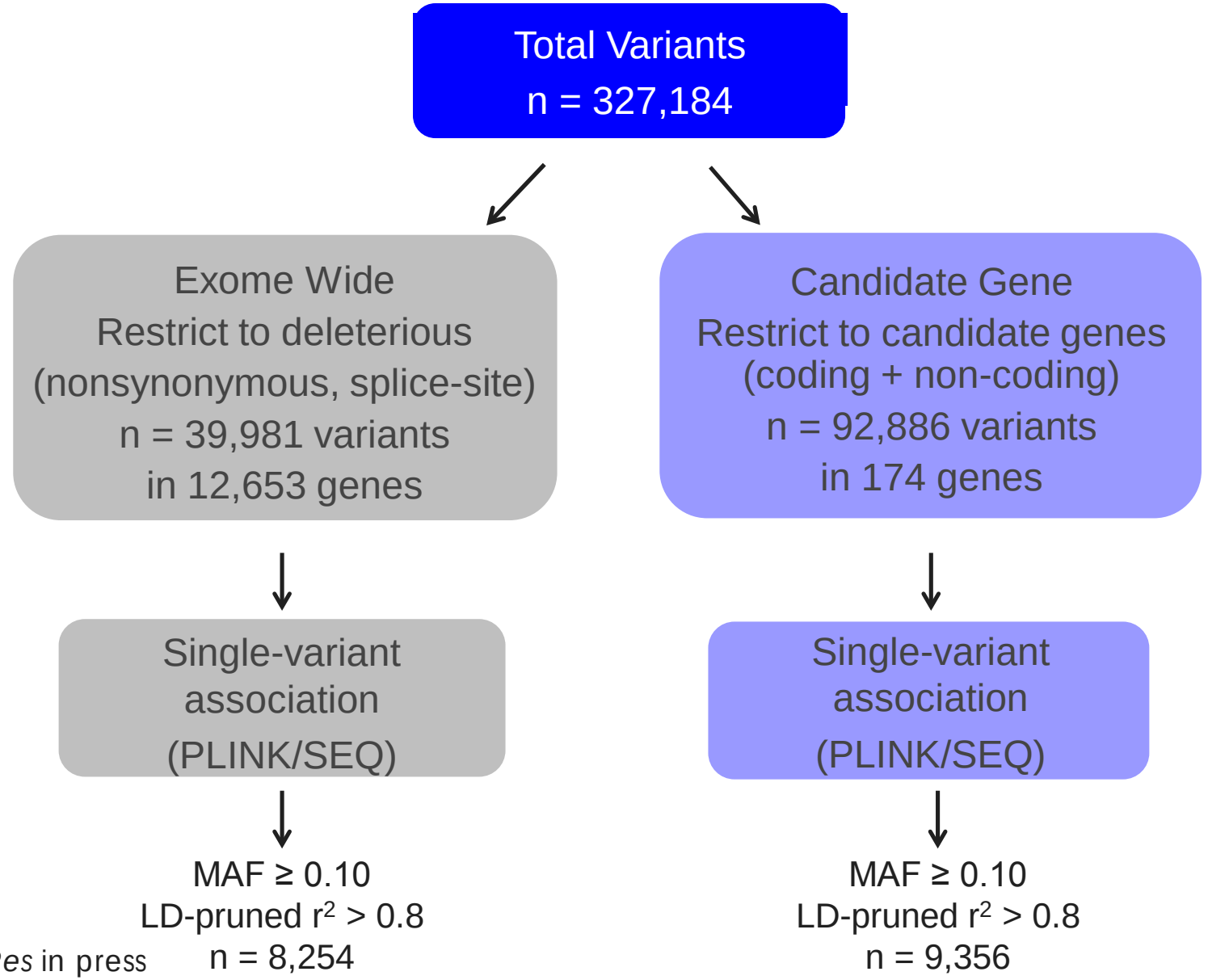
PI Alan Venook

Bevacizumab-Induced Hypertension Cases and Controls



Megan Li *Clin Cancer Res* in press

Variant Association Testing



Megan Li *Clin Cancer Res* in press

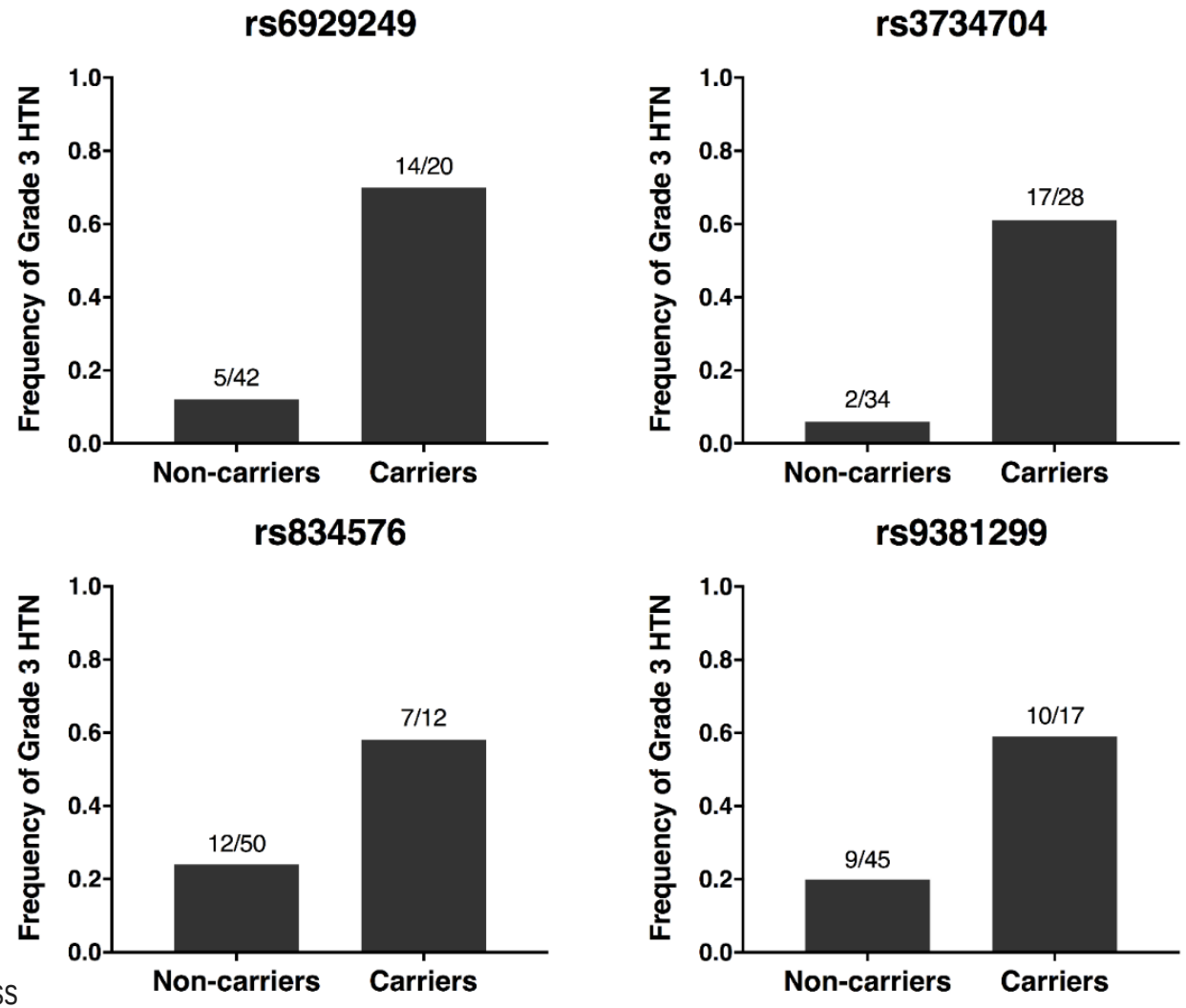
Candidate Gene SNP Analysis of Bevacizumab-Induced Hypertension

rsid	Candidate Gene	Function	P	OR	Case genotypes (MAF)	Control genotypes (MAF)	1000G EUR MAF
rs6929249	*HSP90AB1*	5' upstream	1.8E-04	36.3	5/13/1 (0.39)	37/6/0 (0.07)	0.17
rs3734704	*HSP90AB1*	5' upstream	0.001	24.7	2/14/3 (0.53)	32/10/1 (0.14)	0.27
rs2470417	*CACNA1C*	5' upstream	0.002	11.6	6/12/1 (0.37)	33/9/1 (0.13)	0.22
rs59189065	*PRKCA*	intronic	0.003	9.9	7/11/1 (0.34)	35/8/0 (0.09)	0.20
rs834576	*HSP90AB1*	5' upstream	0.003	12.2	12/6/1 (0.21)	38/5/0 (0.06)	0.05
rs1463664	*RAF1*	3' downstream	0.003	7.3	10/5/3 (0.29)	34/7/0 (0.08)	0.14
rs72869749	*PDE3B*	intronic	0.004	26.2	9/9/1 (0.29)	36/7/0 (0.08)	0.14
rs9381299	*HSP90AB1*	5' upstream	0.004	8.2	9/8/2 (0.32)	36/7/0 (0.08)	0.15
rs142385484	*NOSIP*	3' downstream	0.004	13.8	12/7/0 (0.18)	38/4/1 (0.07)	0.15
rs73057960	*NOSIP*	intronic	0.004	18.9	11/7/1 (0.24)	40/2/1 (0.05)	0.14

Bonferroni-adjusted significance threshold: P = 5.3E-06

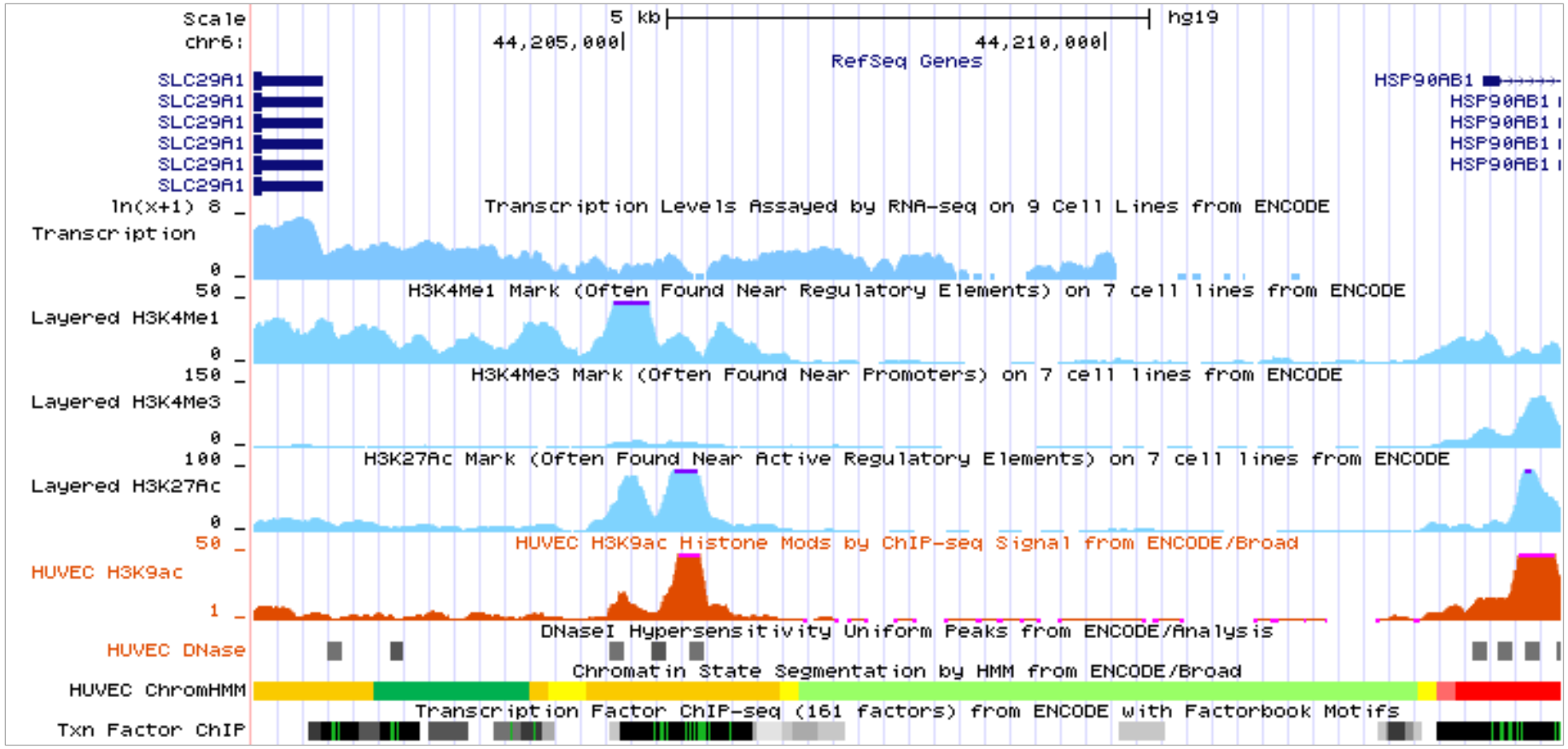
Megan Li *Clin Cancer Res* in press

Carriers of *HSP90AB1* SNPs Have Higher Incidence of Bevacizumab-Induced Grade 3 Hypertension



Megan Li *Clin Cancer Res* in press

SLC29A1-HSP90AB1 Intergenic Region



rs3734704

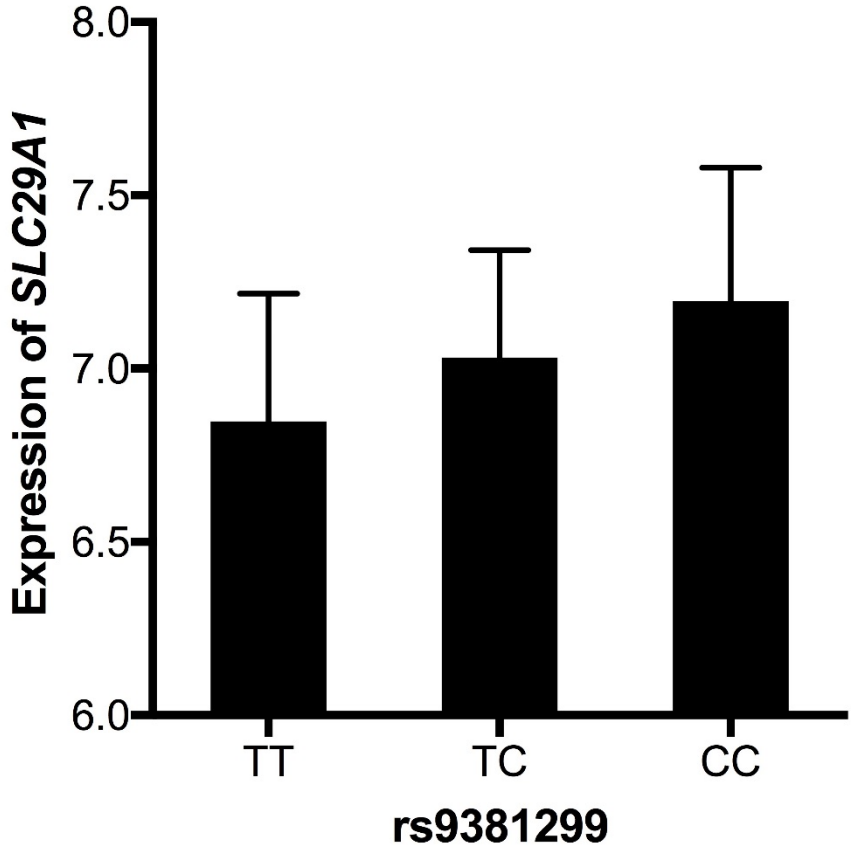
rs6929249: r^2=0.52

rs6929249

rs834576

rs9381299

rs9381299 eqTL: Increased SLC29A1 Expression in Monocytes



P = 3.8 x 10^{-5}, Effect Size = 0.18

Data from Fairfax et al, Nat Genet (2012)

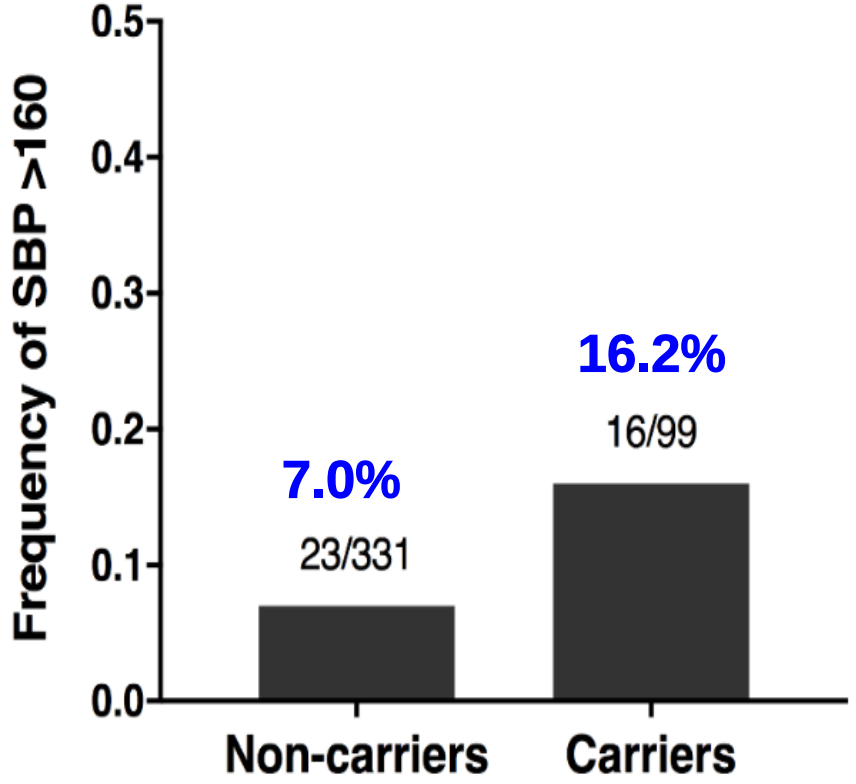
rs9381299 Carriers Have Higher Incidence of Grade 3+ Bevacizumab-Induced Hypertension in Replication Cohorts

CALGB 40502

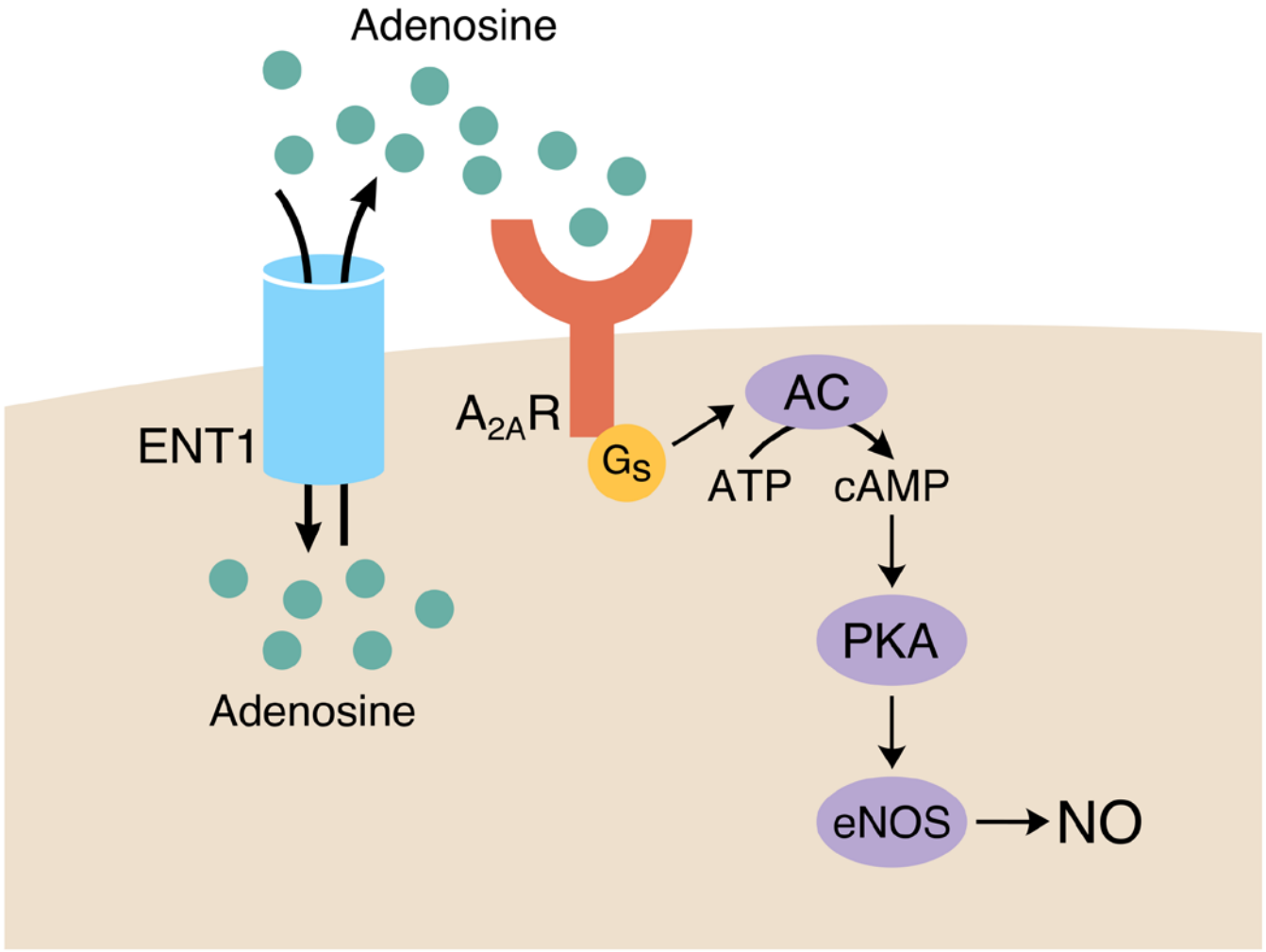
14.3%

4.7%

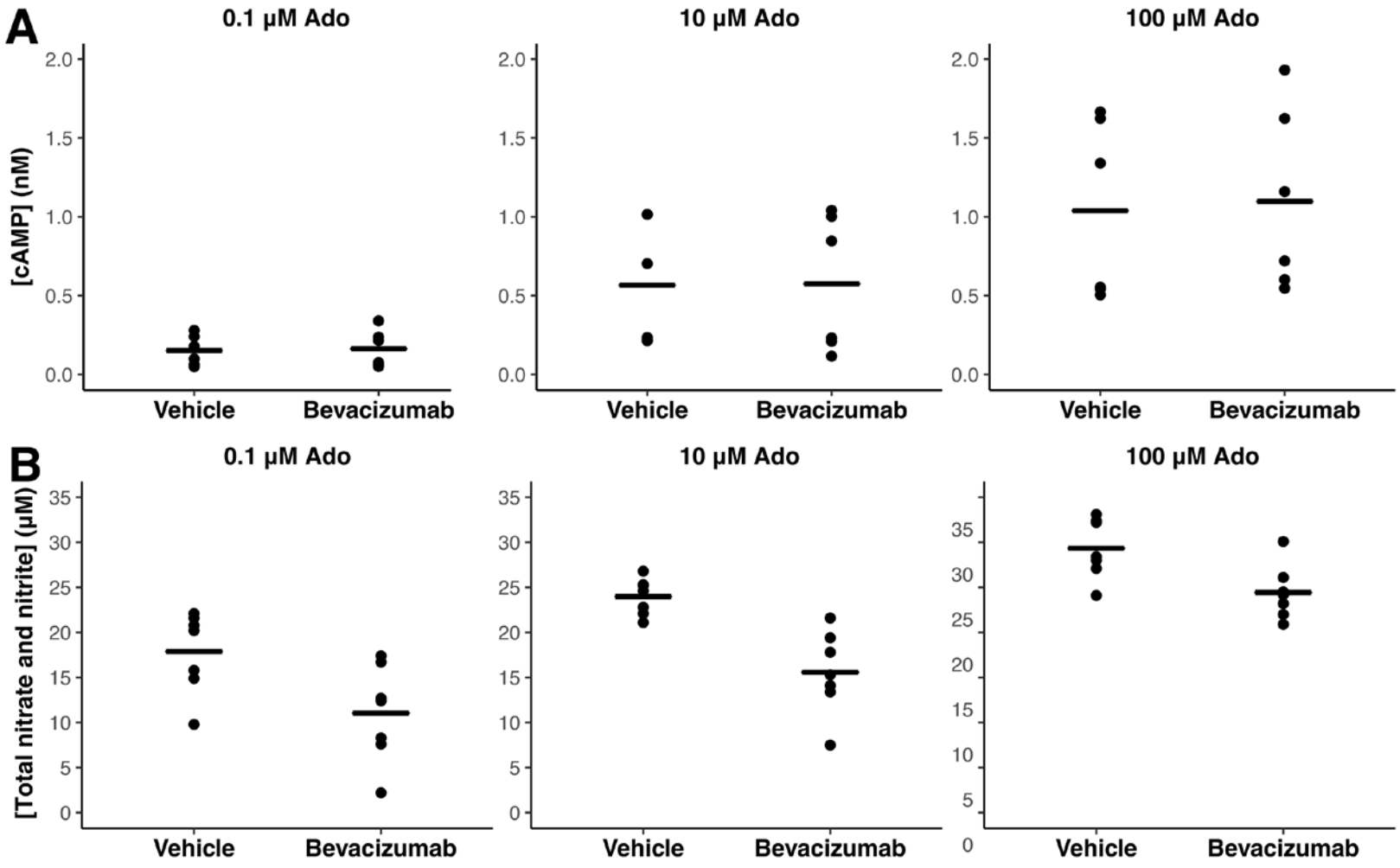
ECOG 5103



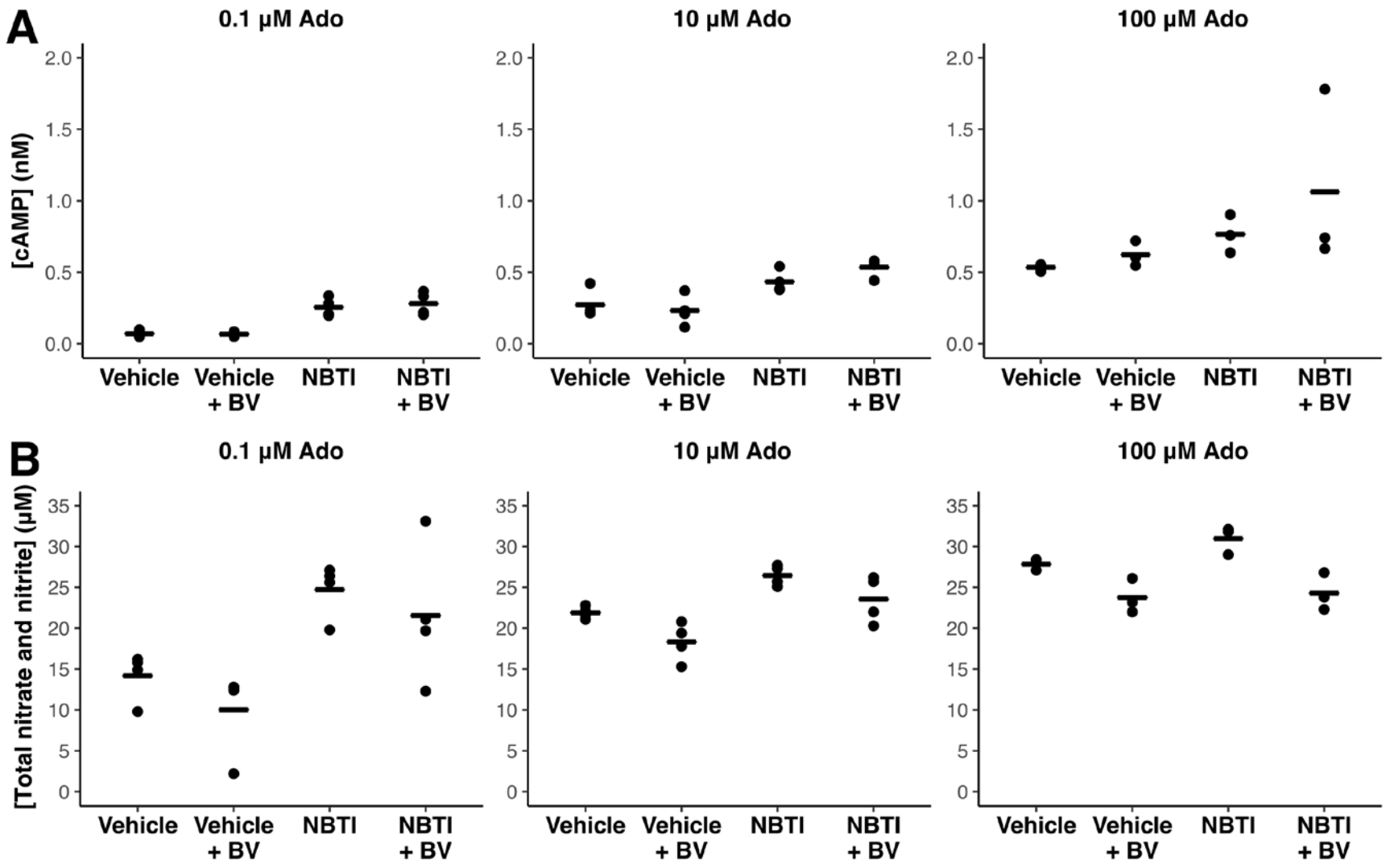
ENT1 (*SLC29A1*) Regulates Adenosine Intra- and Extracellular Levels and Adenosine Receptor Signaling



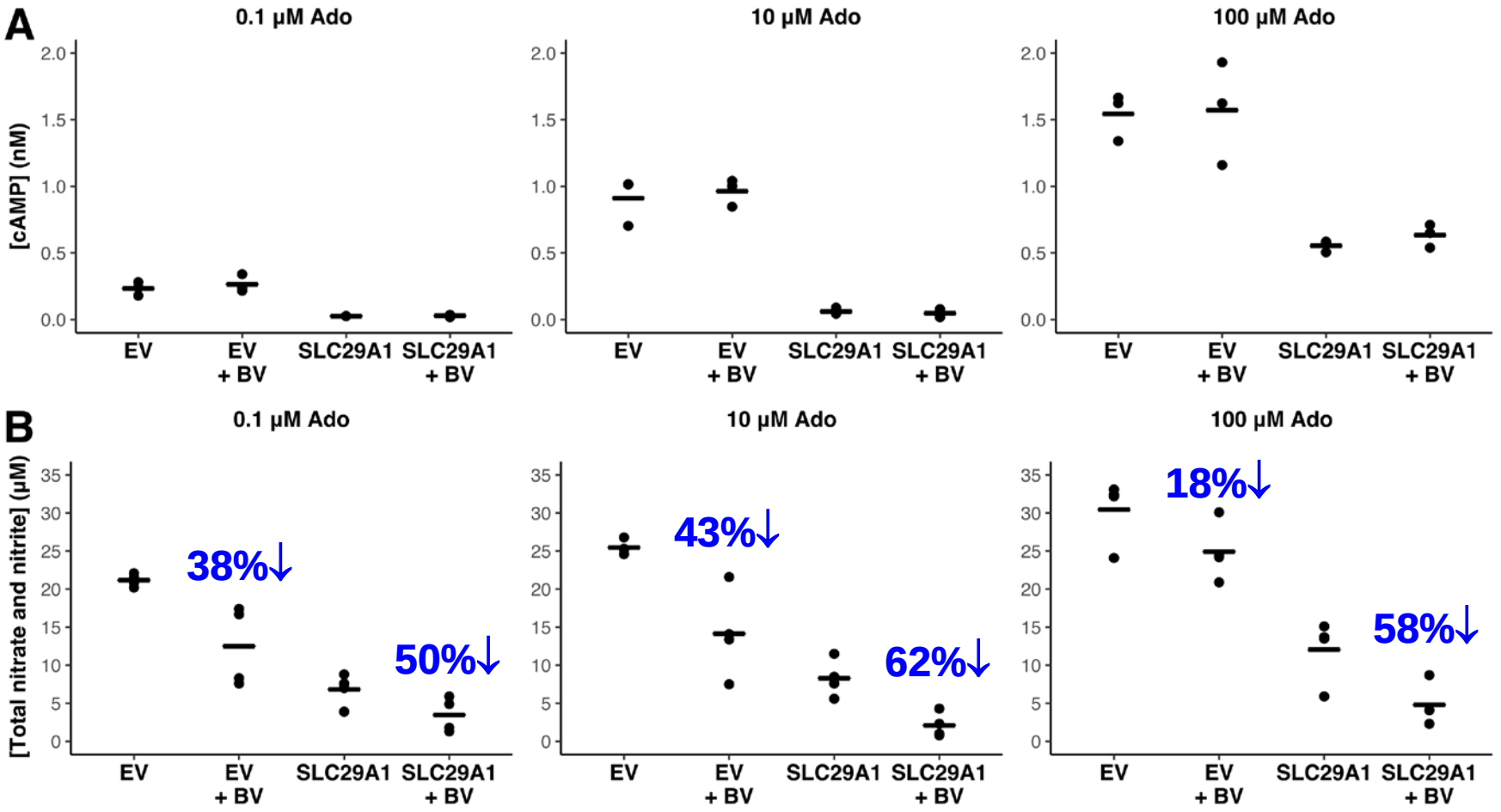
VEGF Signaling but not Adenosine Signaling is Responsive to Bevacizumab Treatment in HUVECs



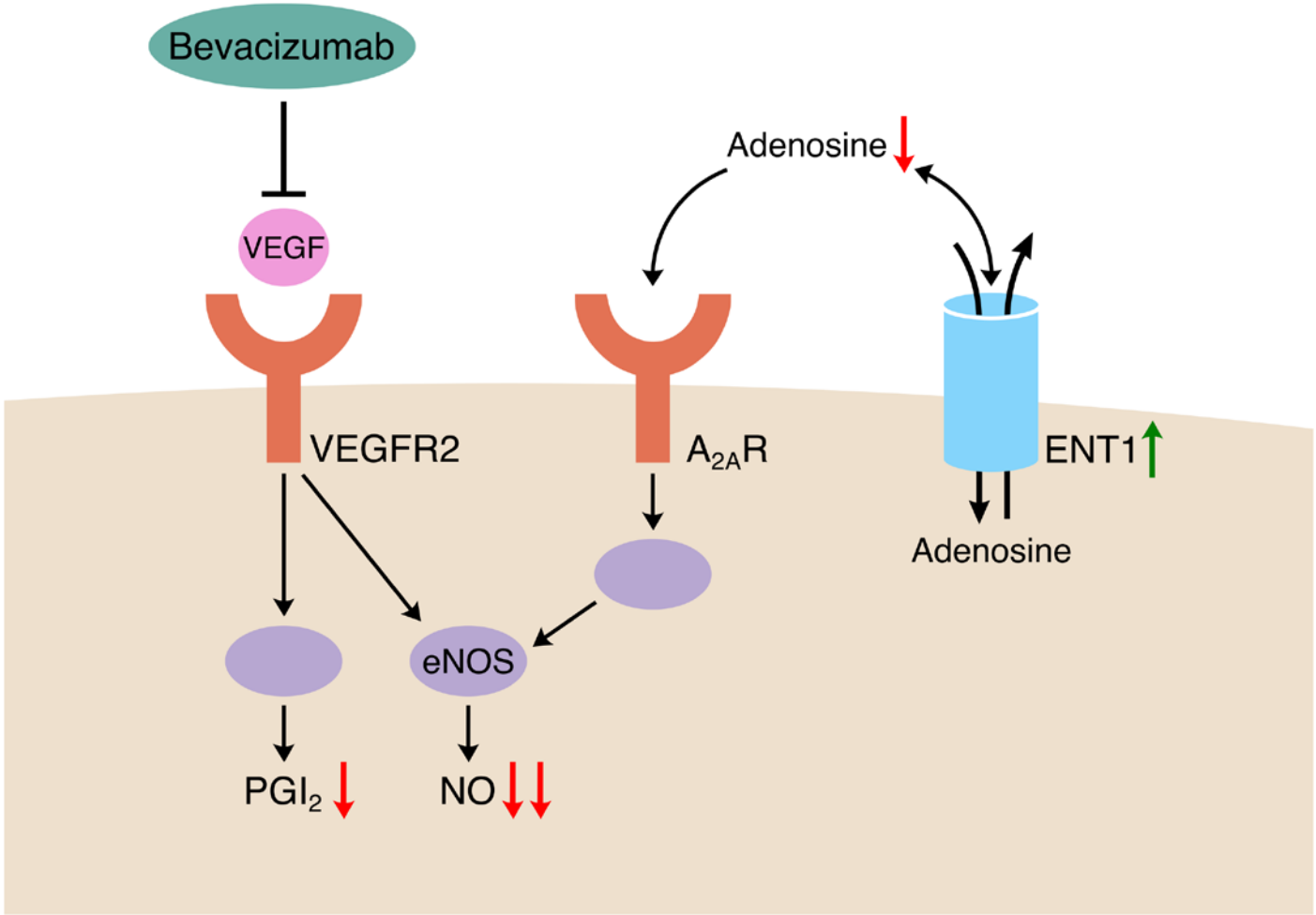
Inhibition of ENT1 Increases Adenosine Signaling in a Bevacizumab-Independent Manner



Overexpression of ENT1 Increases the Response of HUVECs to Bevacizumab



Hypothesis: Endothelial Cells are more Sensitive to VEGF Inhibition Under Conditions of Decreased Basal Adenosine Signaling

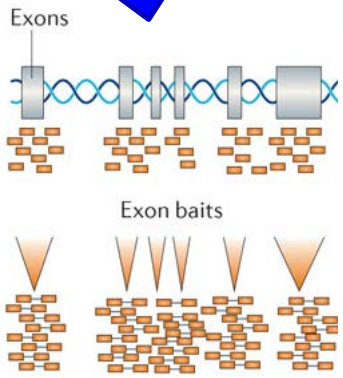


Conclusions and Current Focus

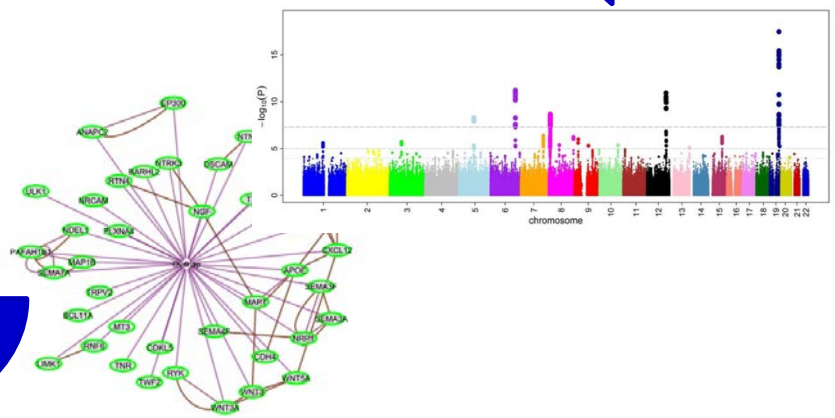
- Variation in adenosine signaling influences vascular response to bevacizumab treatment
- Whether variants in the *SLC29A1-HSP90AB1* genomic region influence gene expression requires further study
- Variation in HSP90AB1 signaling may independently influence the risk of developing bevacizumab-induced hypertension

Reverse Translation of Adverse Drug Events

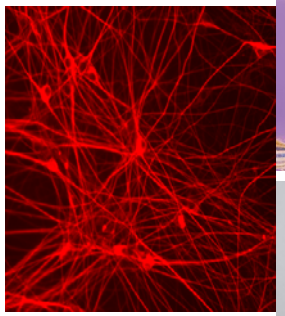
Bedside to Bench



Computational Analysis



Lab Analyses



Bench to Bedside

Acknowledgments

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