Overview

- Background
- 3 studies using MA27 trial
  - Musculo-Skeletal Adverse Events
    - Study Design and Statistical Analysis
    - Pharmacogenomics Functional Studies
  - Bone Fracture : Osteoporosis
    - Study Design and Statistical Analysis
    - Pharmacogenomics Functional Studies
  - Breast Cancer Recurrence
    - Study Design and Statistical Analysis
    - Pharmacogenomics Functional Studies
  - Conclusions and future work

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VOLUME 31 · NUMBER 11 · APRIL 10 2013

JOURNAL OF CLINICAL ONCOLOGY

ORIGINAL REPORT

31:1398-1404, 2013

Exemestane Versus Anastrozole in Postmenopausal Women With Early Breast Cancer: NCIC CTG MA.27—A Randomized Controlled Phase III Trial

Paul E. Goss, James N. Ingle, Kathleen I. Pritchard, Matthew J. Ellis, George W. Sledge, G. Thomas Budd, Manuela Rabaglio, Rafat H. Ansari, David B. Johnson, Richard Tozer, David P. D'Souza, Haji Chalchal, Silvana Spadafora, Vered Stearns, Edith A. Perez, Pedro E.R. Liedke, Istvan Lang, Catherine Elliott, Karen A. Gelmon, Judy-Anne W. Chapman, and Lois E. Shepherd

- Largest trial examining aromatase inhibitors as adjuvant therapy for early stage hormone receptor positive breast cancer (n=7,576 patients)
- No difference between exemestane and anastrozole
- Majority (79.5%, 5,427 of 6827 North American patients) of patients consented to collection and use of DNA for genetic studies

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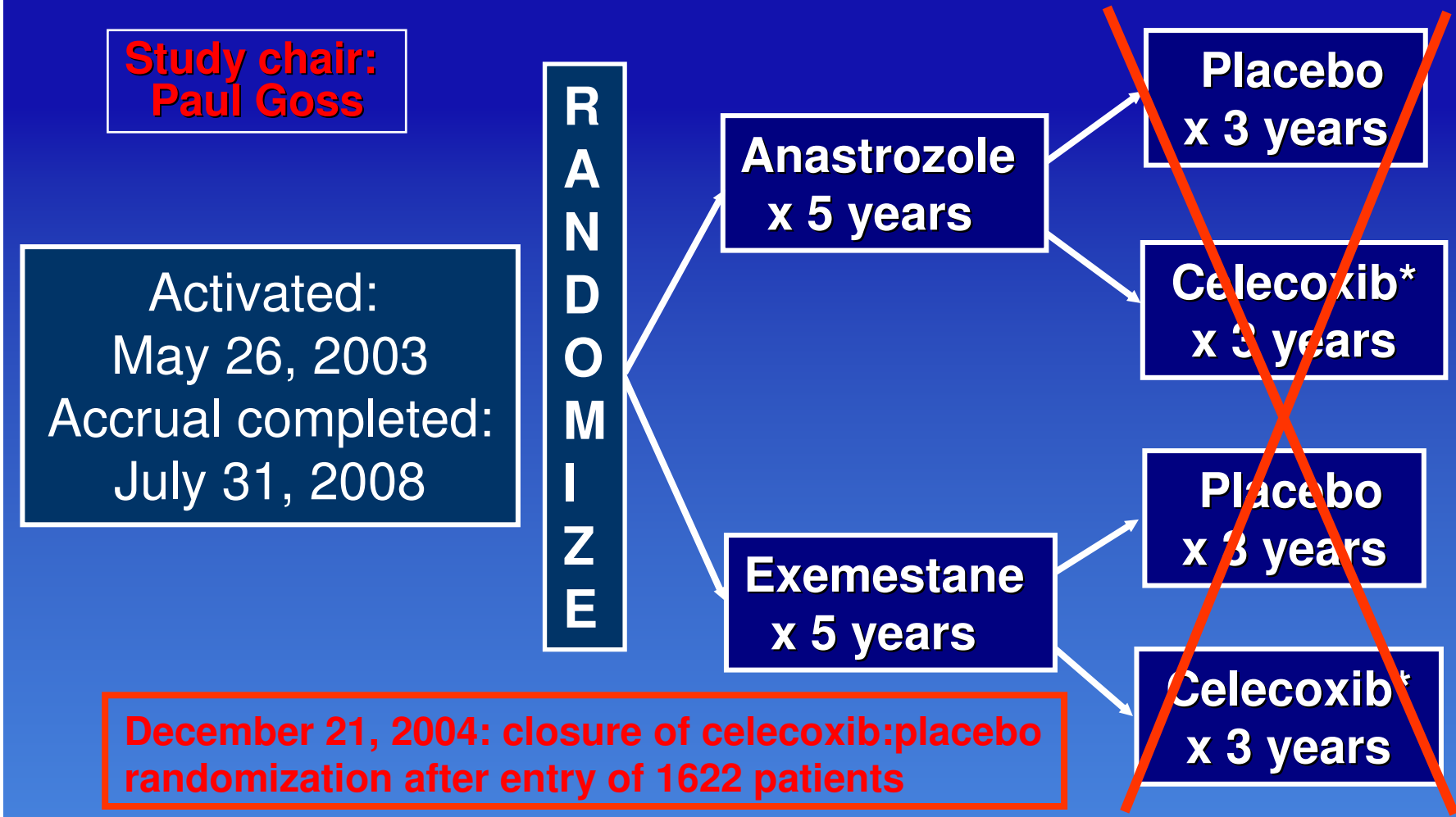
NCIC-CTG TBCI*
Postmenopausal Breast Cancer Adjuvant Trial
MA.27



*The Breast Cancer Intergroup of North America: NCIC CTG, CALGB, ECOG, NCCTG, SWOG

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Introduction

- Aromatase inhibitors (AI)
  - Postmenopausal patients with ER+ breast cancer are treated with AI drugs
- Side effects
  - About one-half of patients have joint-related complaints with AI therapy (Crew, JCO, 2007; 25:3877)
  - Bone Fractures

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Aromatase Inhibitors are important in the management of postmenopausal women with early stage breast cancer

American Society of Clinical Oncology
Clinical Practice Guideline, 2010

"consider incorporating aromatase inhibitor therapy at some point during adjuvant treatment, either as up-front therapy or as sequential treatment after tamoxifen."

J Clin Oncol 28:3784-96, 2010

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AI therapy advantages

- AIs are even more effective than Tam monotherapy in preventing recurrence and breast cancer death

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GWAS and Functional Follow-up of Muscular Skeletal Events

Ingle, J.N., D.J. Schaid, P.E. Goss, M. Liu, T. Mushiroda, J.A. Chapman, M. Kubo, G.D. Jenkins, A. Batzler, L. Shepherd, J. Pater, L. Wang, M.J. Ellis, V. Stearns, D.C. Rohrer, M.P. Goetz, K.I. Pritchard, D.A. Flockhart, Y. Nakamura, and R.M. Weinshilboum, Genome-wide associations and functional genomic studies of musculoskeletal adverse events in women receiving aromatase inhibitors. J Clin Oncol, 2010. **28**(31): 4674-82.

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Hypothesis PGRN-RIKEN-MA.27 Study

A genome-wide association case control study will identify single nucleotide polymorphisms associated with musculoskeletal adverse events (MS-AEs) in women receiving aromatase inhibitor adjuvant therapy for early breast cancer

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Design

- This study was blinded for Treatment arm and Celecoxib allocation
- A nested matched case-control study with two controls for each case. Matching on the following factors:
  - Treatment arm (exemestane vs.anastrozole)
  - Prior chemotherapy (yes/no)
  - Age at treatment (+/- 5 years)
  - Celecoxib allocation (yes/no)
- Restricted to self-identified Caucasians (94% of accrued patients)

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NCI Common Terminology Criteria for Adverse Events (Version 3.0)
Arthralgia

- Grade 1: Mild pain not interfering with function
- Grade 2: Moderate pain; pain or analgesics interfering with function, but not interfering with activities of daily living (ADL)
- Grade 3: Severe pain; pain or analgesics severely interfering with ADL
- Grade 4: Disabling

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Case Selection

- Case definition: grade 3-4 MS-AE or off-treatment for *any* grade of MS-AE
- MS-AE must occur within the first two years
- Exclude from the case group subjects who met the case definition while on celecoxib or in the three months after stopping celecoxib
- Available DNA and consent

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Control Selection

- No report of any grade MS-AE
- Followed six months longer than the matched case
- Off celecoxib for at least six months

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Patient Characteristics

		Cases (n=293)	Controls (n=585)
Age	Median	63.3	64.1
	Range	46.1-86.9	45.1-84.4
Treatment, %	A	56	56
	B	44	44
Prior chemo, %	No	68	69
	Yes	32	31
Celecoxib, %	C	75	73
	D	25	28
Prior HRT, %	Unknown	7	6
	No	35	53
	Yes*	65	47
BMI at baseline**	Median	28.2	27.9
	Range	17.7-56.8	16.9-50.8

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* extended Fisher's exact test, p<0.001

** 291 cases, 577 controls

Genotype Quality Control and SNPs for Analyses

- Call Rates: 906 of 912 (99.3%) samples (cases, controls, duplicates, CEPH trios) with call rate >0.98
- Received genotyping data on 580,955 SNPs
- In pool of cases and controls, MAF < 1% in 29,478 SNPs (removed from analysis)
- Hardy-Weinberg in controls, $P < 10^{-6}$
  82 SNPs (removed from analysis)
- Number of SNPs in analyses:
  580,955 – 29,478 – 82 = 551,395

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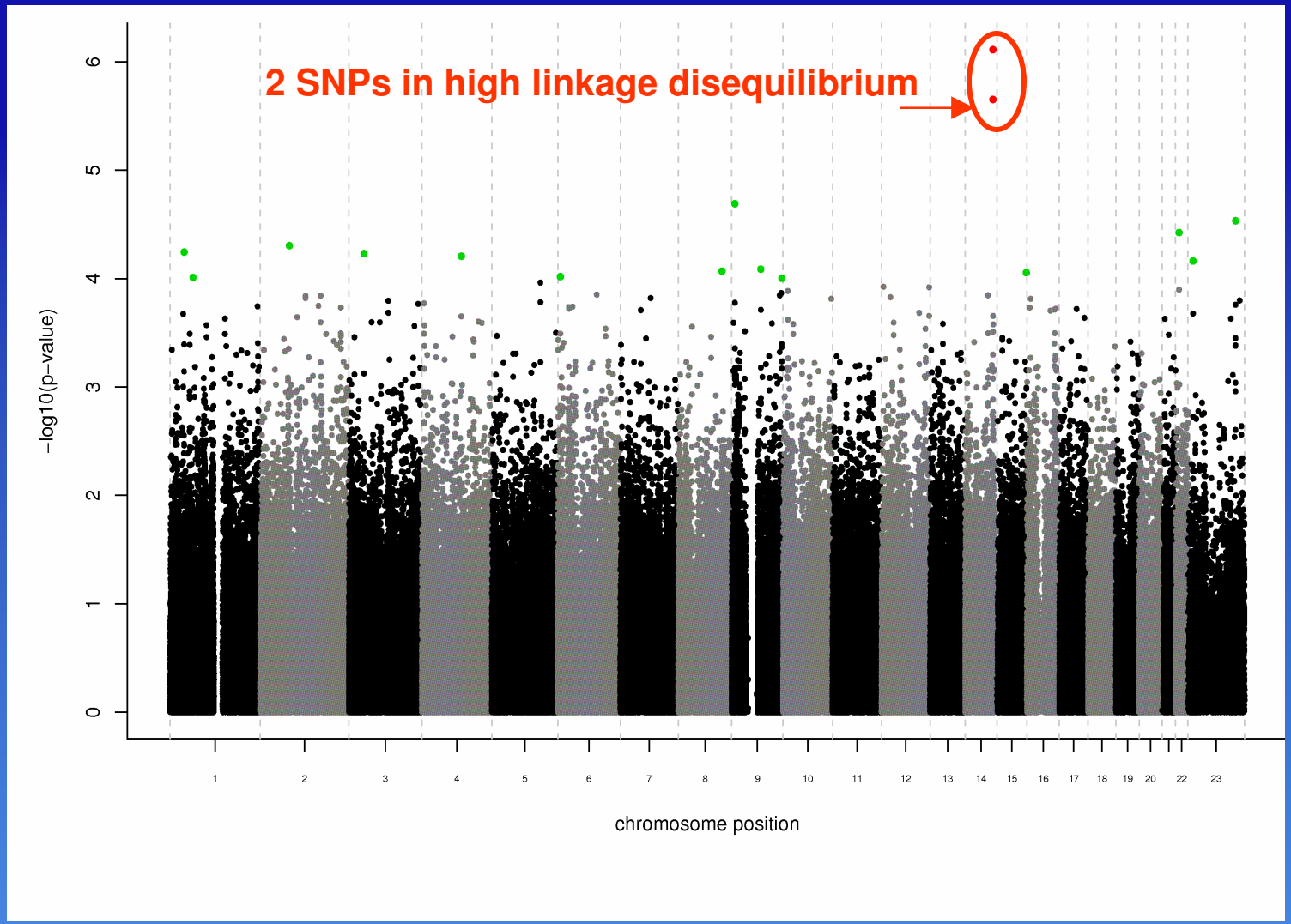
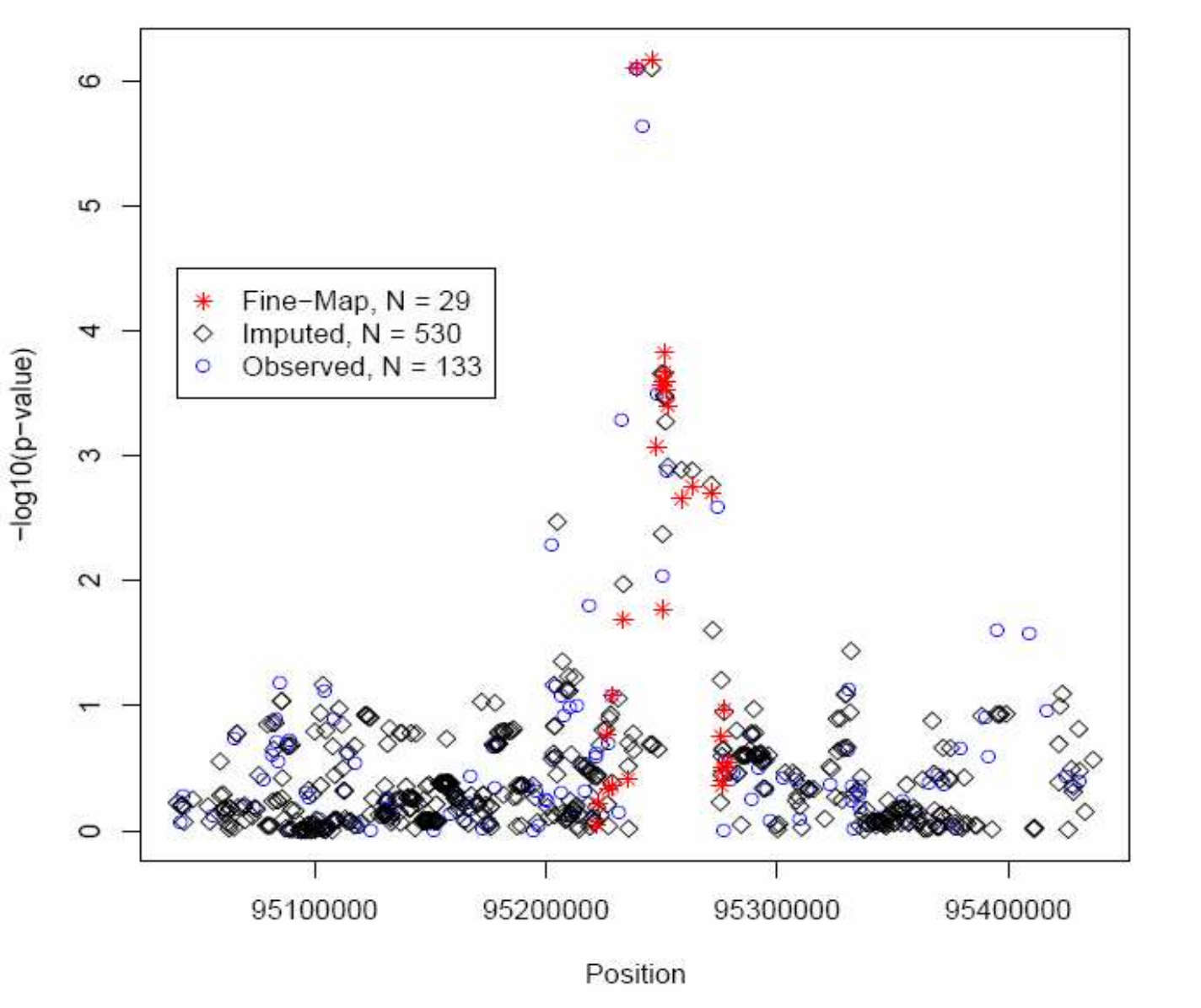
Conditional Logistic regression adjusted for 8 Eigenvectors



*adjusted for 8 eigenvectors

Fine mapping of +/- 200 kb region



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Imputation & Fine mapping

- SNPs were imputed within 300 kb of the smallest P value on Chr 14 showed an additional SNP
  - MACH 1.0 with white CEPH European Ref panel
- Fine mapping within 200kb region of the imputed data was done on 29 SNPs
- Based on LD we picked 20kb region including the 4 SNPs of interest.
- Re-sequencing did not find SNPs with stronger association than rs11849538 (70 dbSNPs & 40 novel).

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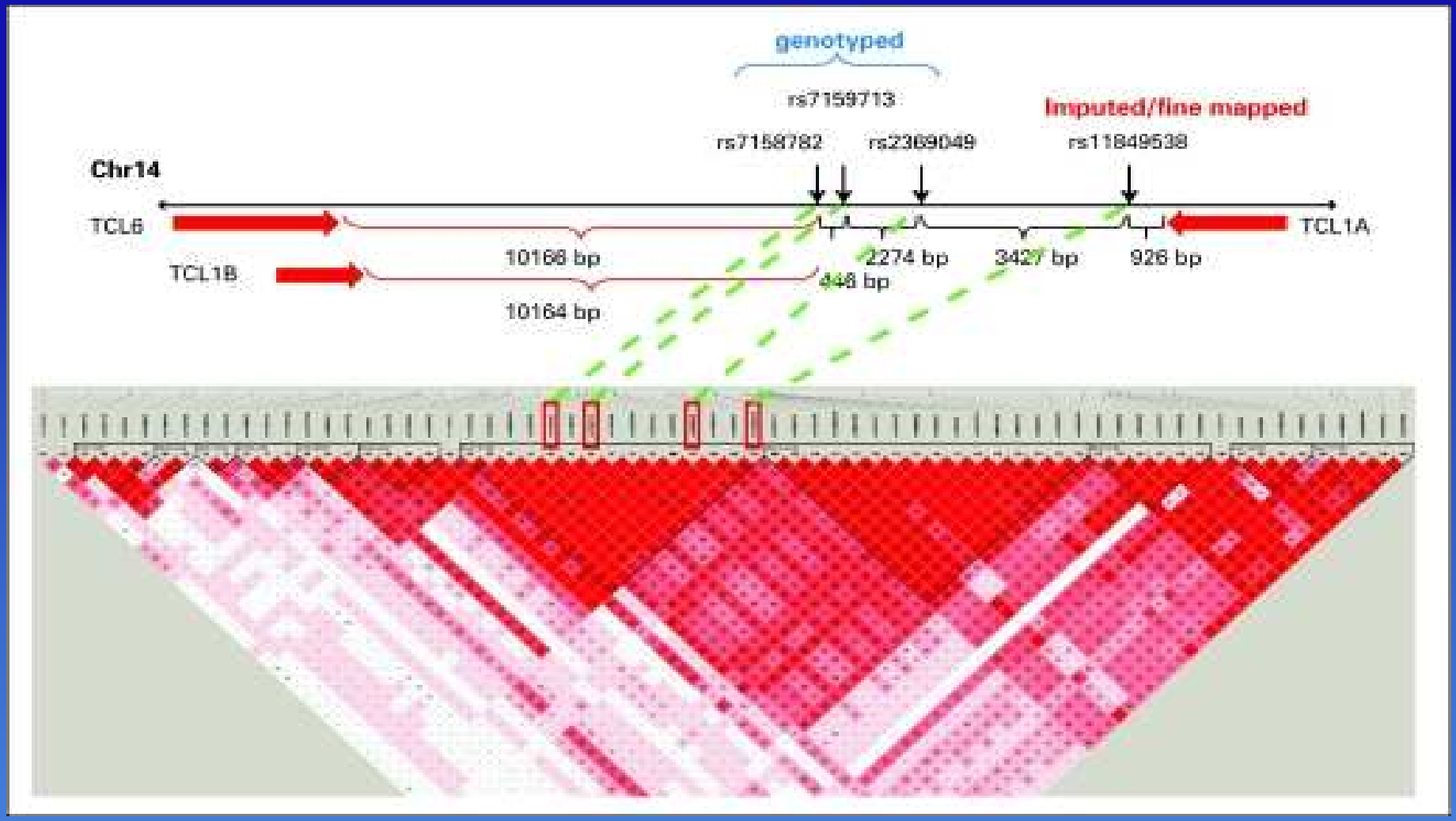
SNPs with Lowest P values

SNP	MAF		OR	P-Value	Type
	Cases	Controls			
rs11849538*	0.172	0.091	2.21	6.67E-07	Imputed & Finemapped
rs7158782	0.190	0.110	2.16	7.74E-07	Genotyped
rs7159713	0.190	0.110	2.16	7.74E-07	Genotyped
rs2369049	0.180	0.100	2.08	2.23E-06	Genotyped

Chromosme 14, MA.27 GWAS signal



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Challenges

- SNP function
- Relating SNPs to genes
- Relating genes to drug effect

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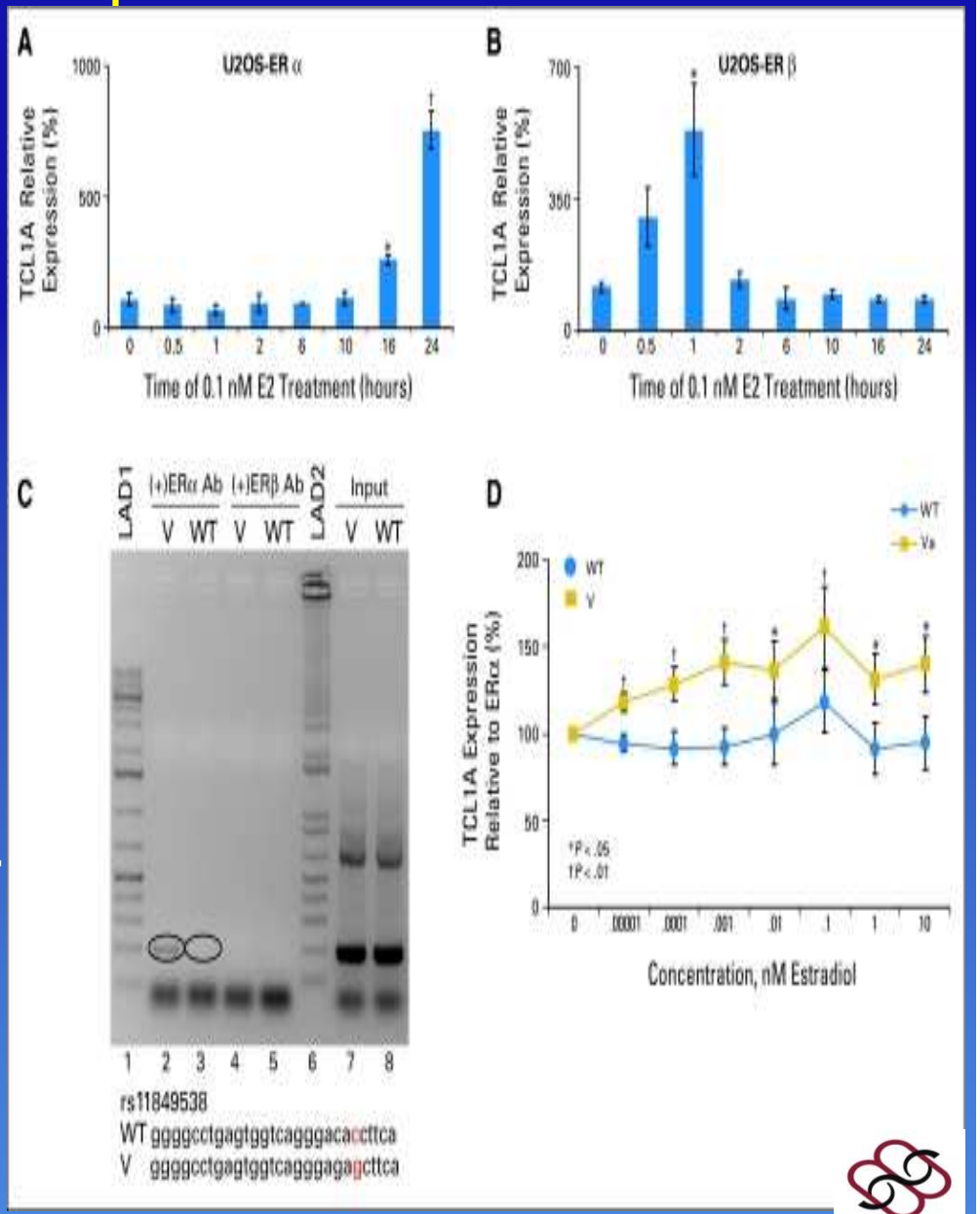
Estrogen induced TCL1A expression variation

- E2 induces *TCL1A* expression in U2OS cells transfected with ERα or ERβ
- Most significant SNP (rs11849538) creates an estrogen response element
- Lymphoblastoid cells transfected with ERα: *TCL1A* expression greater for variant than wild type



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Pharmacogenomics Model System

"Human Variation Panel"
300 LCL Cell Lines

- 100 EA, 100 AA, 100 HCA
- 1.3 million SNPs/cell line (~7 million after imputation)
- 54,000 expression array probes/cell line
- Genome-wide CpG methylation

Liewei Wang, M.D., Ph.D.

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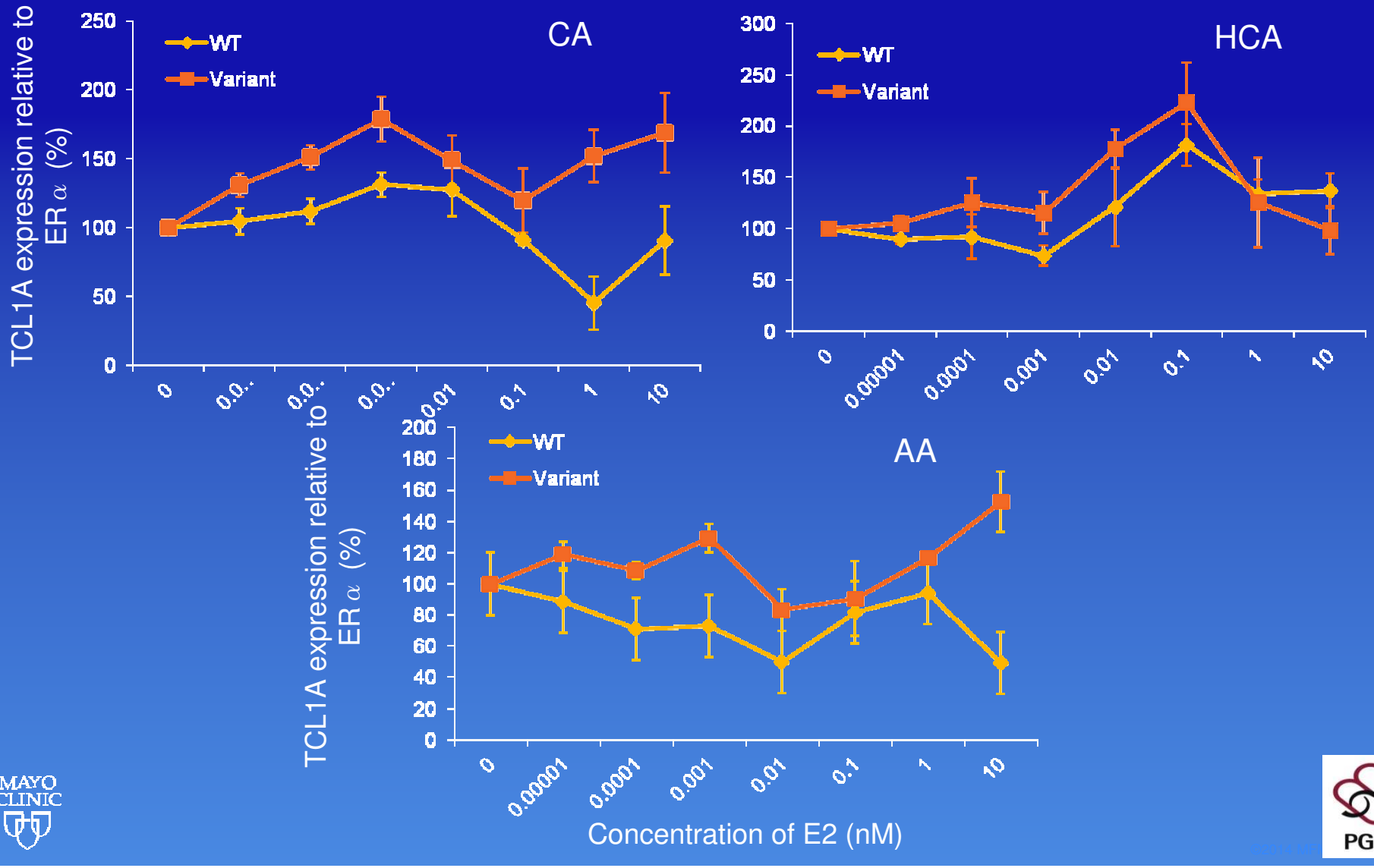
SNP-related Differences in TCL1A Expression to Estrogen Response in Three Ethnic Groups in ERα-Transfected "Human Variation Panel" Cells



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Conclusions

- This GWAS identified 4 SNPs in linkage disequilibrium on Chr14 associated with musculoskeletal adverse events in women receiving aromatase inhibitors
- These SNPs appear to be functionally significant based on EMSA, ChIP assays and their association with *TCL1A* expression
- Women with a musculoskeletal adverse event after AI therapy are more likely to have a variant on Ch14 that creates an ERE for ERα
- WT and variant SNP sequences had differing effects on the estrogen-dependent expression of *TCL1A*

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GWAS and Functional Follow-up of Fragility Fractures

Liu, M., P.E. Goss, J.N. Ingle, M. Kubo, Y. Furukawa, A. Batzler, G.D. Jenkins, E.E. Carlson, Y. Nakamura, D.J. Schaid, J.A. Chapman, L.E. Shepherd, M.J. Ellis, S. Khosla, L. Wang, and R.M. Weinshilboum, Aromatase Inhibitor-Associated Bone Fractures: A Case-Cohort GWAS and Functional Genomics. Mol Endocrinol, 2014. **28**(10): 1740-51.

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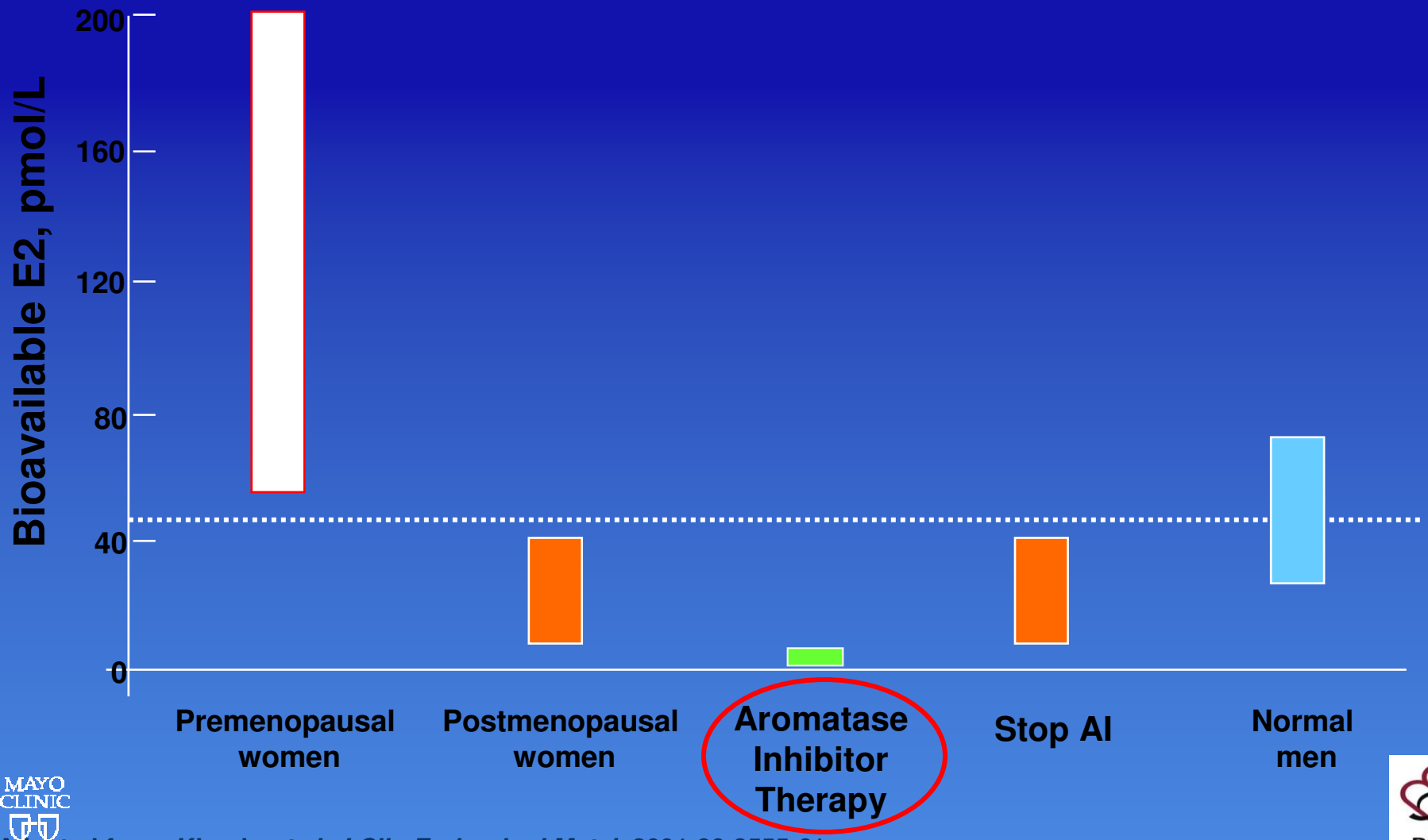
Estrogen Levels in Women and Men



Adapted from: Khosla *et al. J Clin Endocrinol Metab* 2001;86:3555-61

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Primary objective of GWAS

- To identify genetic variation as measured by SNPS associated with fragility fractures in women treated with aromatase inhibitors as adjuvant therapy for early stage breast cancer
- Note: this is not an osteoporosis study

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Definition of Fragility Fracture

Sites of fractures that would be expected to be related to AI-associated bone loss, specifically those in the

- spine
- forearm
- humerus
- proximal femur/hip

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Rationale for Pharmacogenomic study of Bone Fractures in MA.27

- There is a direct relationship between serum estrogen concentrations and osteoporosis risk
- AIs greatly decrease serum estrogen levels in post menopausal women
- Bone loss with clinical fracture is a potentially life-threatening adverse event of AI therapy
- Identifying those at risk for clinical fractures would improve the therapeutic index of AIs

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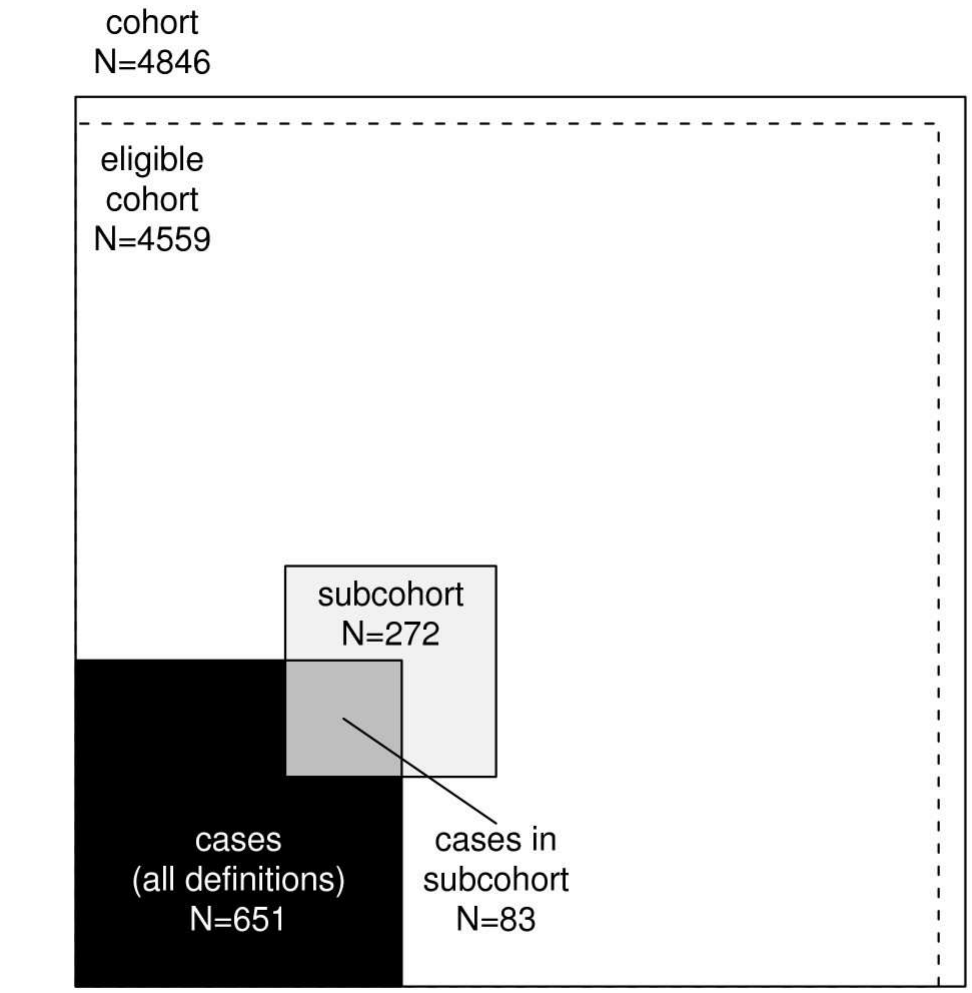
Example Case-Cohort Sampling



case–cohort set for genotyping:
cases ∪ subcohort
N=840

genotyping of:
(1) a random subcohort selected independent of definition of cases
(2) all cases outside the subcohort,

union of (1) and (2) = case-cohort

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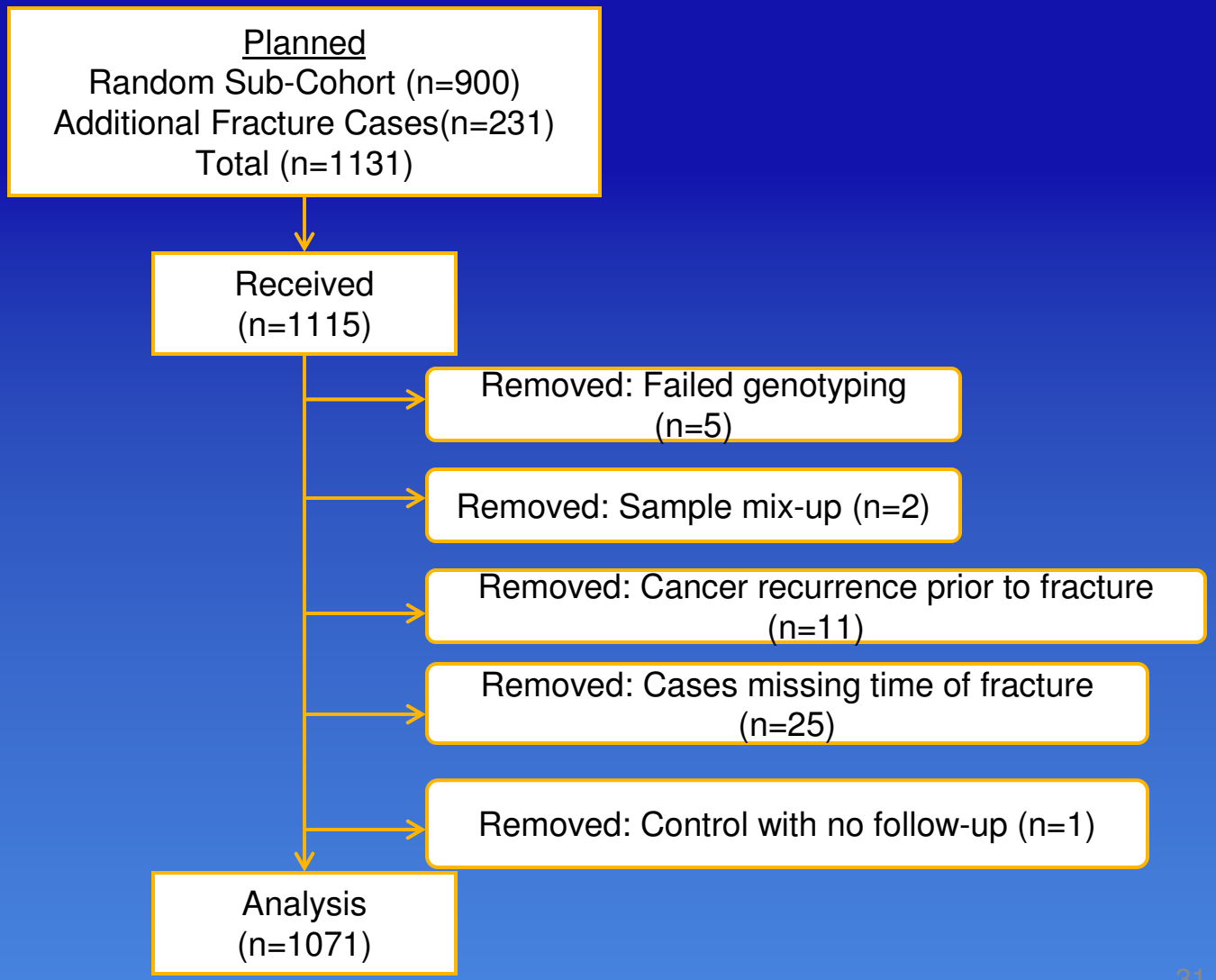
Selection of Subjects for Analyses



Patient Characteristics		
	Cases (N=231)	Controls (N=840)
Age: Med (Range)	68.7 (46.1 – 89.8)	64.2 (35.9 – 88.9)
Prior Fracture (10 yrs)	45 (19.5%)	82 (9.8%)
Prior Chemotherapy	57 (24.7%)	255 (30.4%)
BMI	N=227	N=836
Median (Range)	28.6 (17.4 – 66.8)	28.4 (16.5 – 61.3)
RACE		
Asian	2 (0.9%)	12 (1.4%)
Black	5 (2.2%)	22 (2.6%)
Hawaiian or Pacific Islander	0 (0.0%)	1 (0.1%)
Unknown	0 (0.0%)	3 (0.4%)
White	224 (97.0%)	802 (95.5%)

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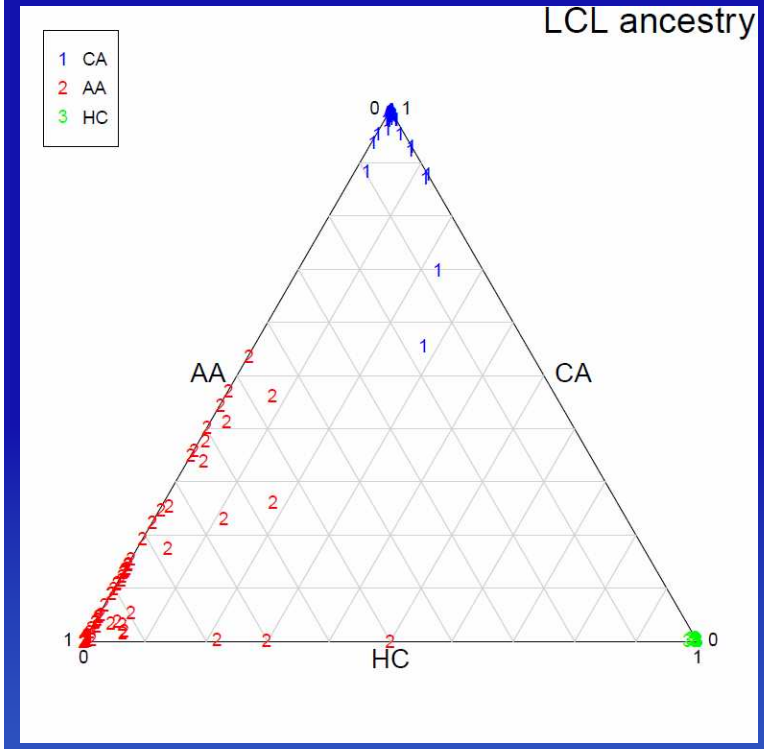
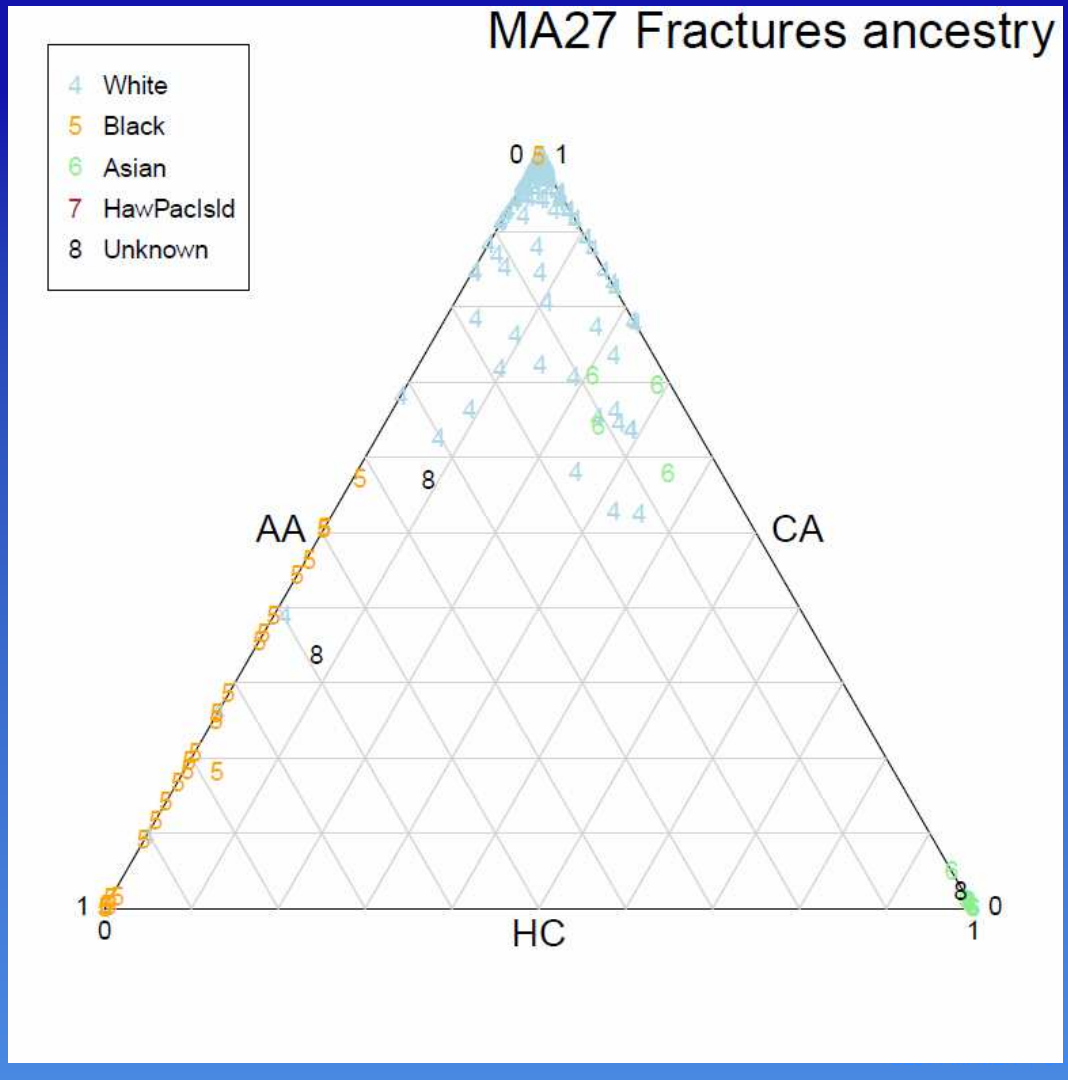

LCL ancestry
1 CA
2 AA
3 HC
AA
CA
HC
0
1


MA27 Fractures ancestry
4 White
5 Black
6 Asian
7 HawPacIsld
8 Unknown
AA
CA
HC
0
1

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Methods: Genotyping

- 887 (83%) on Omni chip
- 184 (17%) on Human610 Quad Beadchip (previously genotyped in AI MS-AE GWAS)

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Screening covariates one at a time

	RelRisk	p-value
Treatment Exemestane	0.994	0.970
chemoYes	0.755	0.094
age.65(65,89.8]	2.273	0.000
ECOG	1.386	0.038
surgeryPartial Mastectomy	1.006	0.967
riken1Yes	0.774	0.206
FracPriorYes	2.351	0.000
RaloxUseYes	1.016	0.977
BisphosUseYes	2.400	0.000
bmi	1.006	0.657
stageTNMII	1.197	0.259
stageTNMIII	1.558	0.102
EVEC.1	0.098	0.376

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Statistical Analysis

- Primary covariates:
  - age
  - Baseline BMI
  - Bisphosphonate use
  - First 3 eigenvectors
- Primary analysis based on a weighted Cox proportional hazard model to account for the case-cohort design
- SNP genotypes analyzed as log-additive effects on risk of an event

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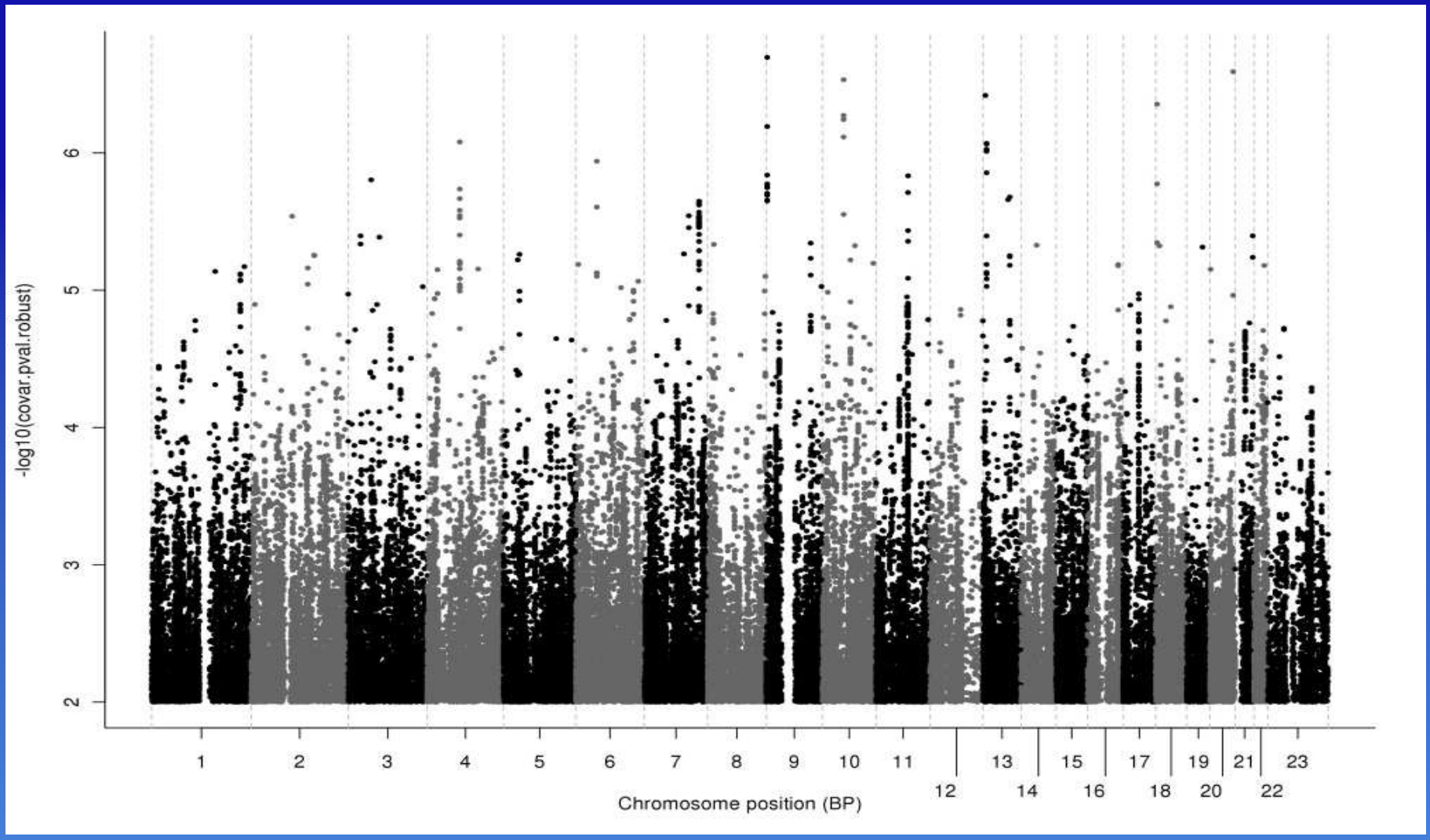
Adj for Clinical & Eigenvec, MAF > .01
(Observed + Imputed: N=7,560,631)



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Validation Decision Cascade

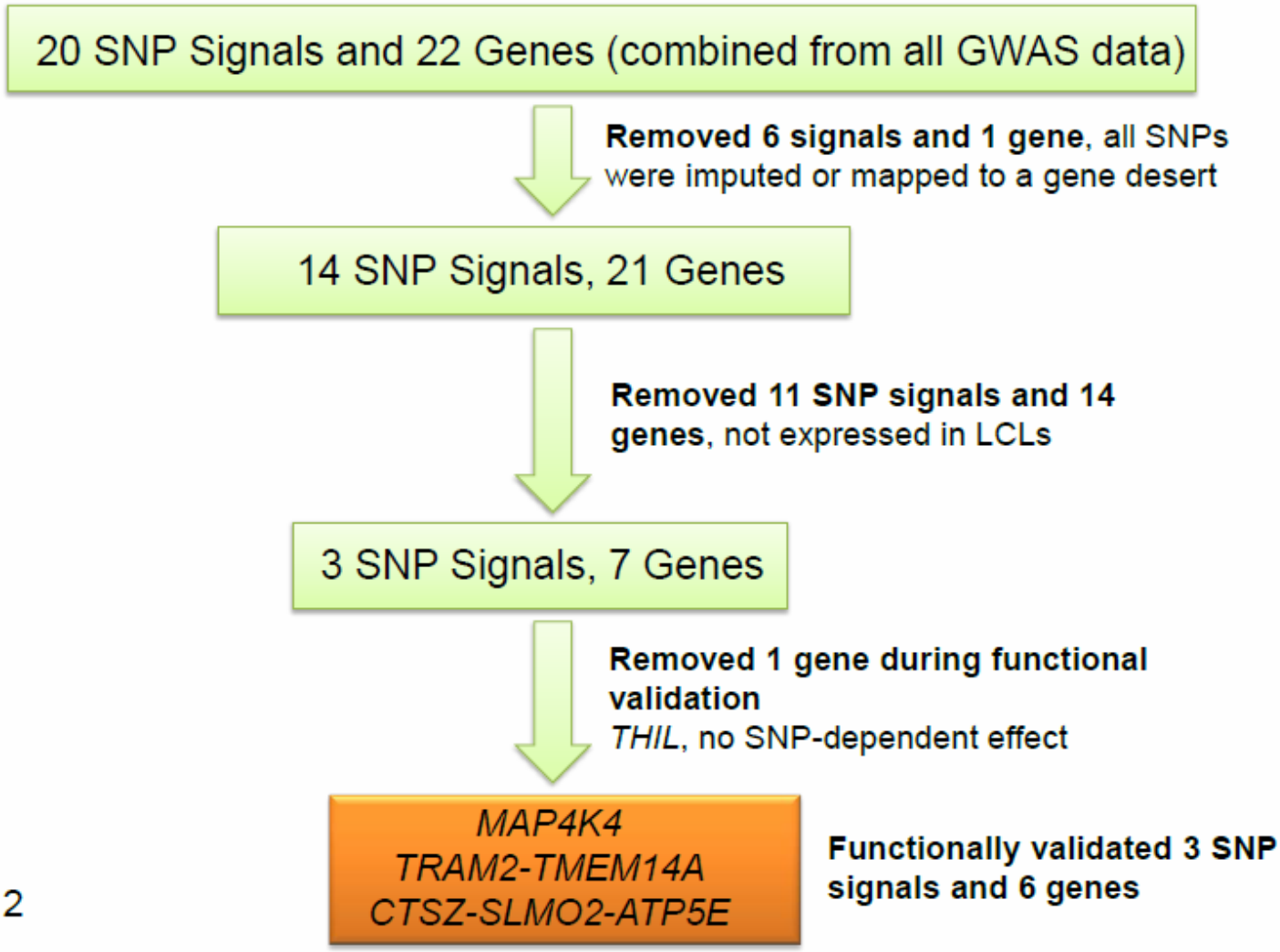

Figure 2

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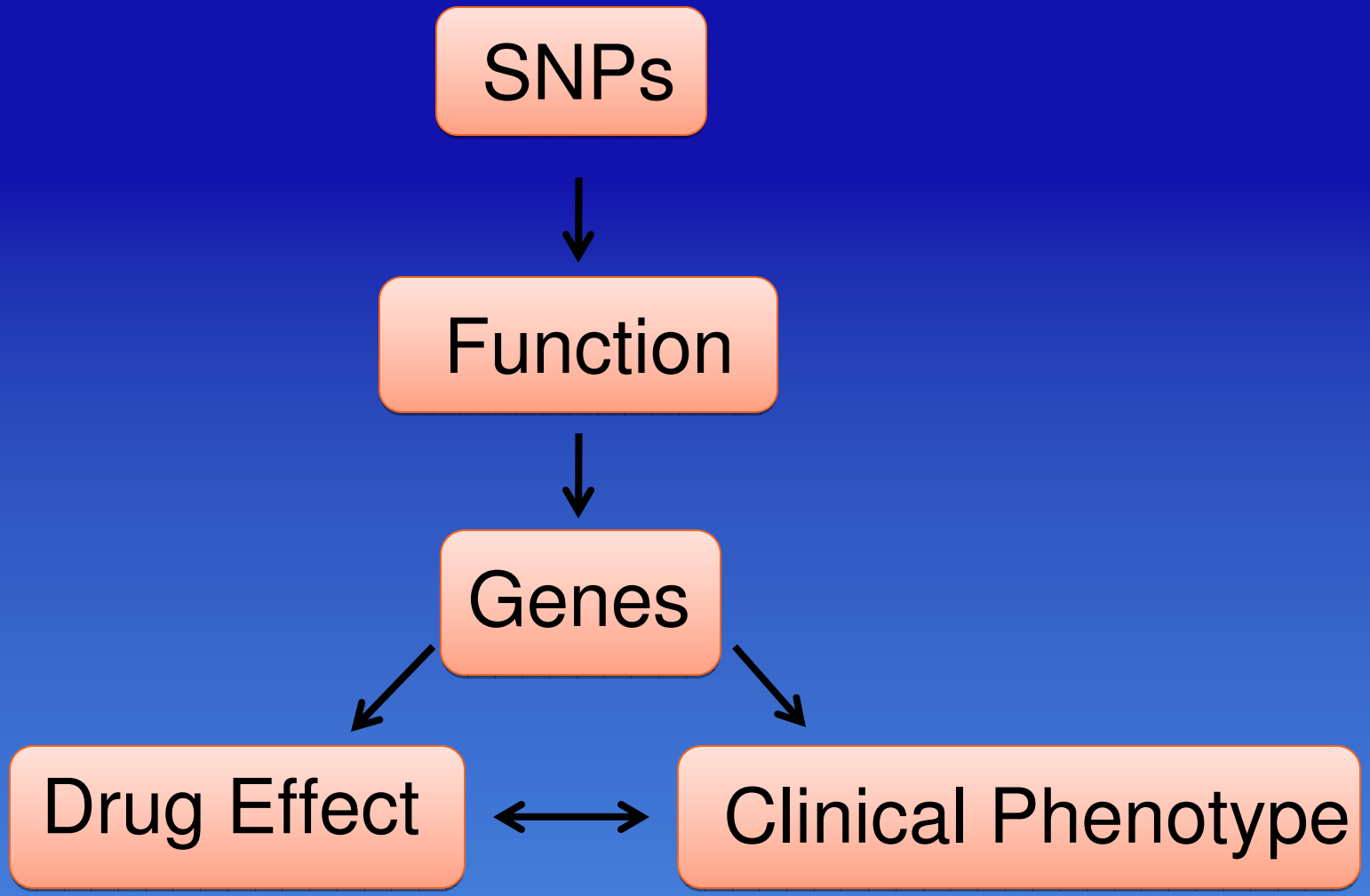
Challenges



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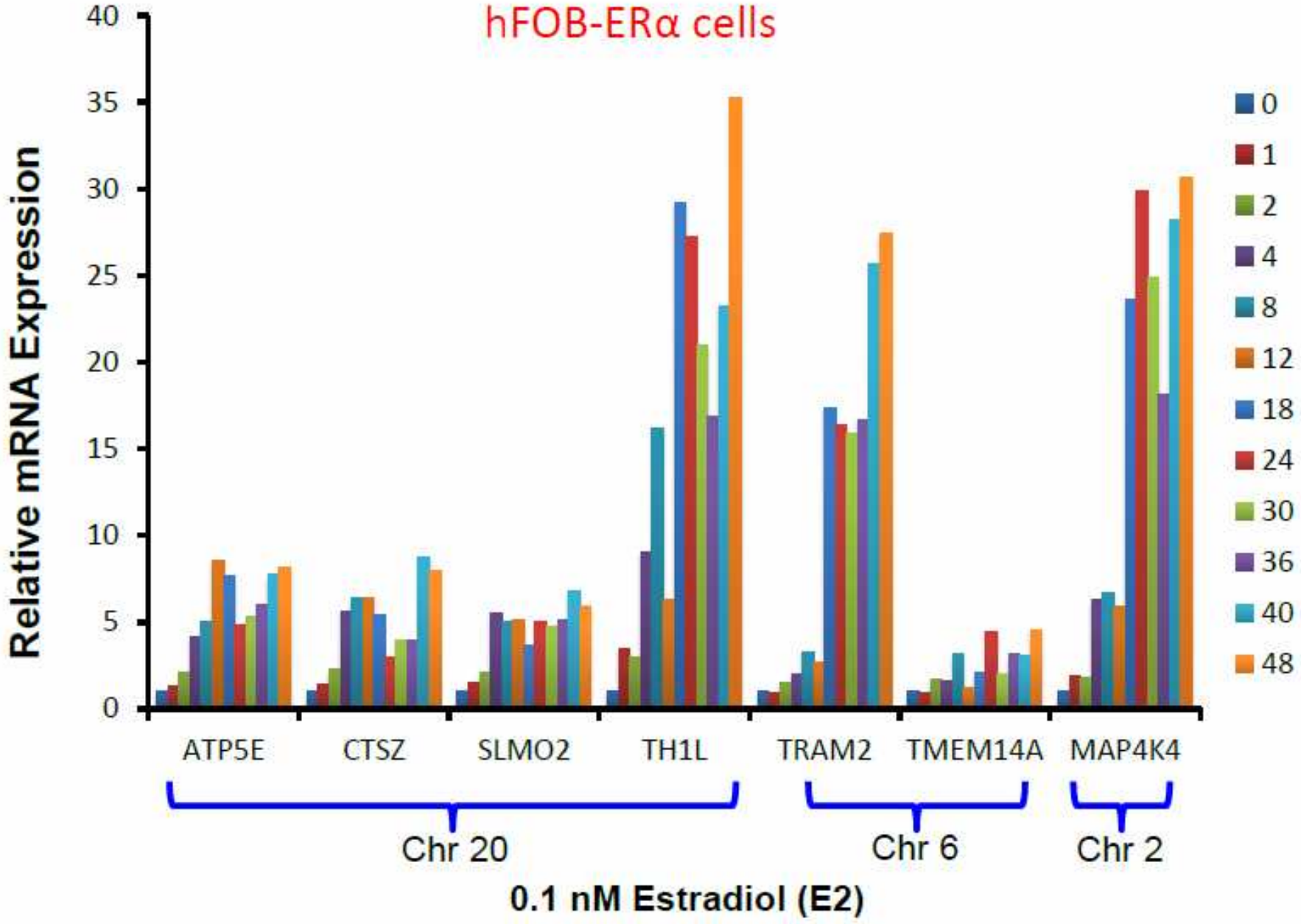

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ARTICLES

nature genetics

Estrada K, et al. Nat Genet 2012; 44 (5):491-501.

Genome-wide meta-analysis identifies 56 bone mineral density loci and reveals 14 loci associated with risk of fracture

- Meta-analysis on lumbar spine and femoral neck BMD
- 17 GWAS involving 32,961 individuals of European and east Asian ancestry
- Top BMD-associated markers tested in 50,933 independent subjects, and
- For association with risk of low-trauma fracture in 31,016 cases (with fracture) and 102,444 controls

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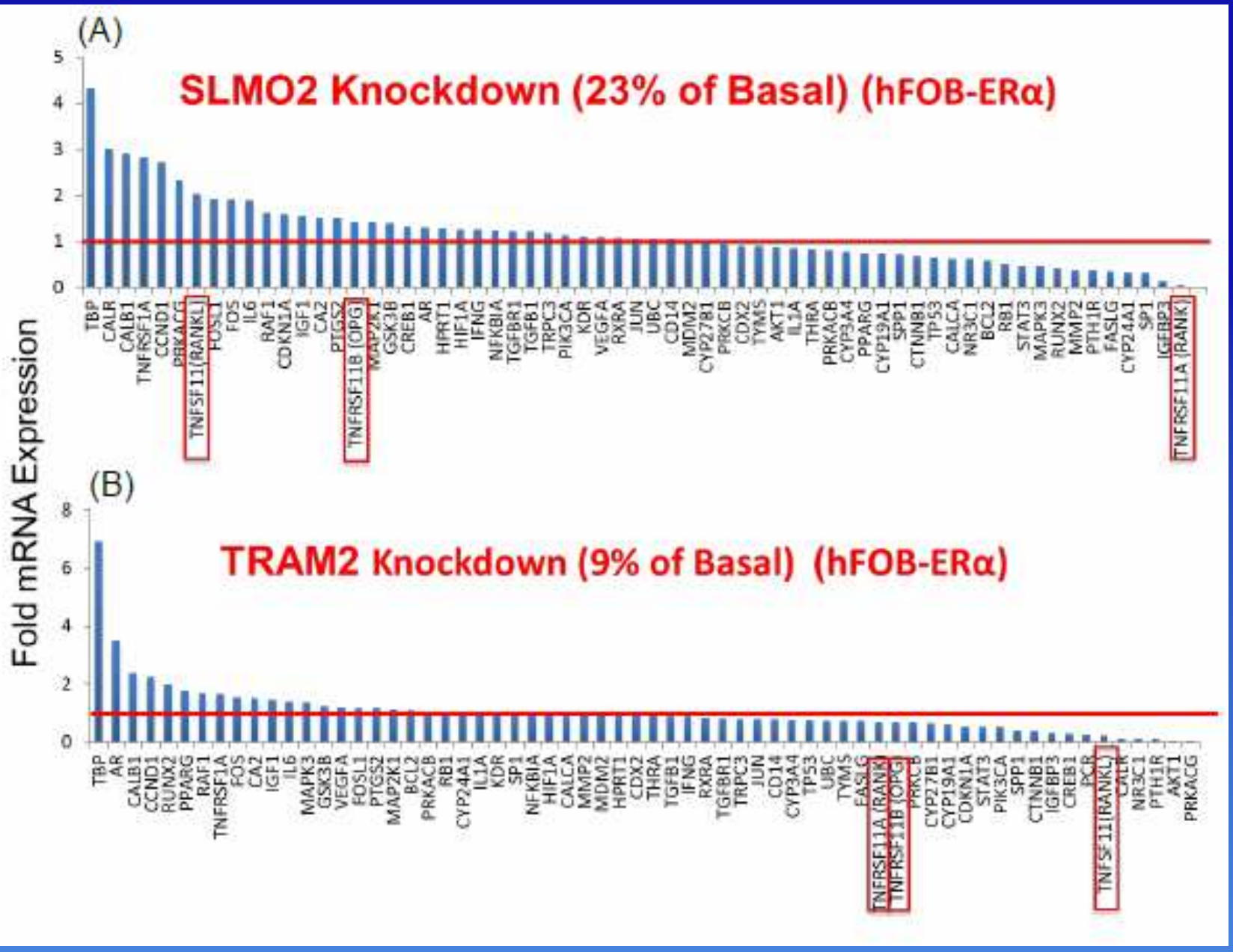

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MA.27 GWAS Gene Expression Correlated with Expression in LCLs of Published Osteoporosis GWAS Genes

	Genes MA.27 GWAS	Genes Osteoporosis GWAS	r	p value
Chr 1	LMNA	CRTAP	0.358	6.77E-10
	LMNA	SLC25A13	-0.26	1.02E-05
	LMNA	SPTBN1	-0.337	6.63E-09
	LMNA	MARK3	-0.274	3.23E-06
Chr 6	MANEA	SPTBN1	0.302	2.43E-07
	MANEA	SLC25A13	0.333	1.10E-08
	MANEA	CRTAP	0.375	8.20E-11
Chr 11	FXC1	SPTBN1	-0.265	6.88E-06
	FXC1	MARK3	0.297	3.94E-07
Chr11	ARFIP2	TNFRSF11A	-0.38	4.46E-11
	ARFIP2	SLC25A13	-0.427	6.72E-14
	ARFIP2	SPTBN1	-0.459	4.59E-16
	ARFIP2	CRTAP	-0.314	7.55E-08
	ARFIP2	PPIB	0.409	9.44E-13
Chr 11	SLC36A4	SPTBN1	-0.296	4.27E-07
	SLC36A4	CRTAP	0.277	2.36E-06

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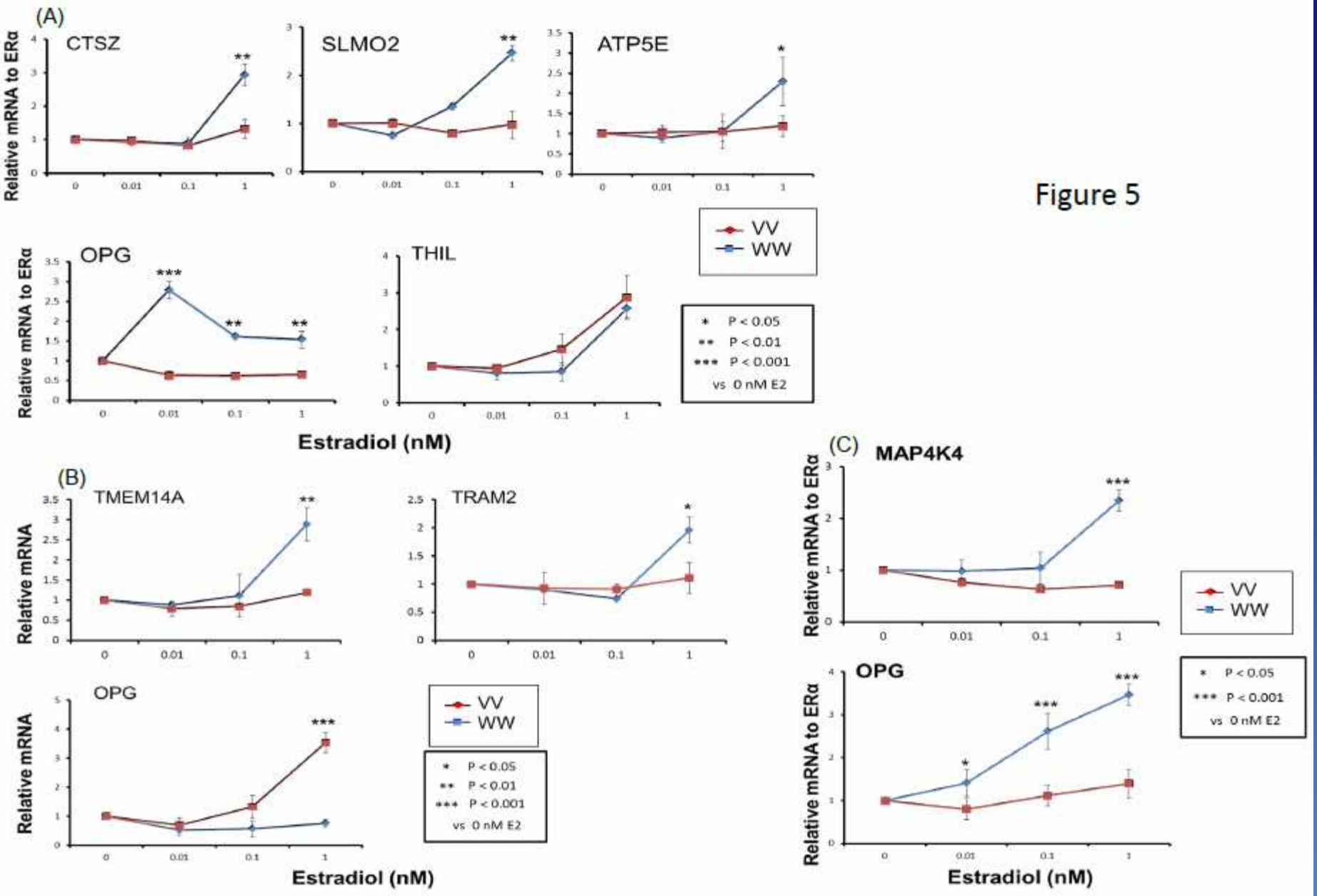
(A)
CTSZ
SLMO2
ATP5E
OPG
THIL
Relative mRNA to ERα
Estradiol (nM)
VV
WW
* P < 0.05
** P < 0.01
*** P < 0.001
vs 0 nM E2
(B)
TMEM14A
TRAM2
OPG
Relative mRNA
Estradiol (nM)
VV
WW
* P < 0.05
** P < 0.01
*** P < 0.001
vs 0 nM E2
(C)
MAP4K4
OPG
Relative mRNA to ERα
Estradiol (nM)
VV
WW
* P < 0.05
*** P < 0.001
vs 0 nM E2

Figure 5

MAF Values of SNPs in Candidate Genes

	Human Variation Panel MAF			1000 Genomes Data MAF		
	AA	CA	HCA	African	European	Asian
MANEA	22%	0	23%	29%	6%	14%
LMNA	49%	0	23%	54%	2%	15%
FXC1/AR FIP2	20%	0	0	26%	1.8%	0
SLC36A4	26%	20%	35%	23%	23%	45%

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Conclusions

- The four genes observed during our fracture GWAS were related to osteoporosis gene expression after estrogen exposure in a SNP-dependent fashion
- The SNPs identified have very small MAFs in Whites (the focus of our GWAS) but were common variants in African Americans and Han Chinese.
- Further study of our “Fracture SNPs and genes” in Blacks and Asians is indicated
- These findings may provide novel insights into the biology of osteoporosis

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GWAS and functional follow-up of Breast Events in MA27 study

Breast Events GWAS

- **Primary objective:** To identify SNPs related to time to a breast event (BCFI) in women receiving aromatase inhibitors on MA.27

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Breast Events GWAS

Patients in GWAS from 3 cohorts of patients entered on MA.27

1.MS-AE GWAS: 843 pts genotyped with Human610 Quad BeadChip

2.Fractures GWAS: 887 pts genotyped on Omni in 2012

3.Breast Events GWAS: 2,927 pts genotyped on OmniExpress in 2013

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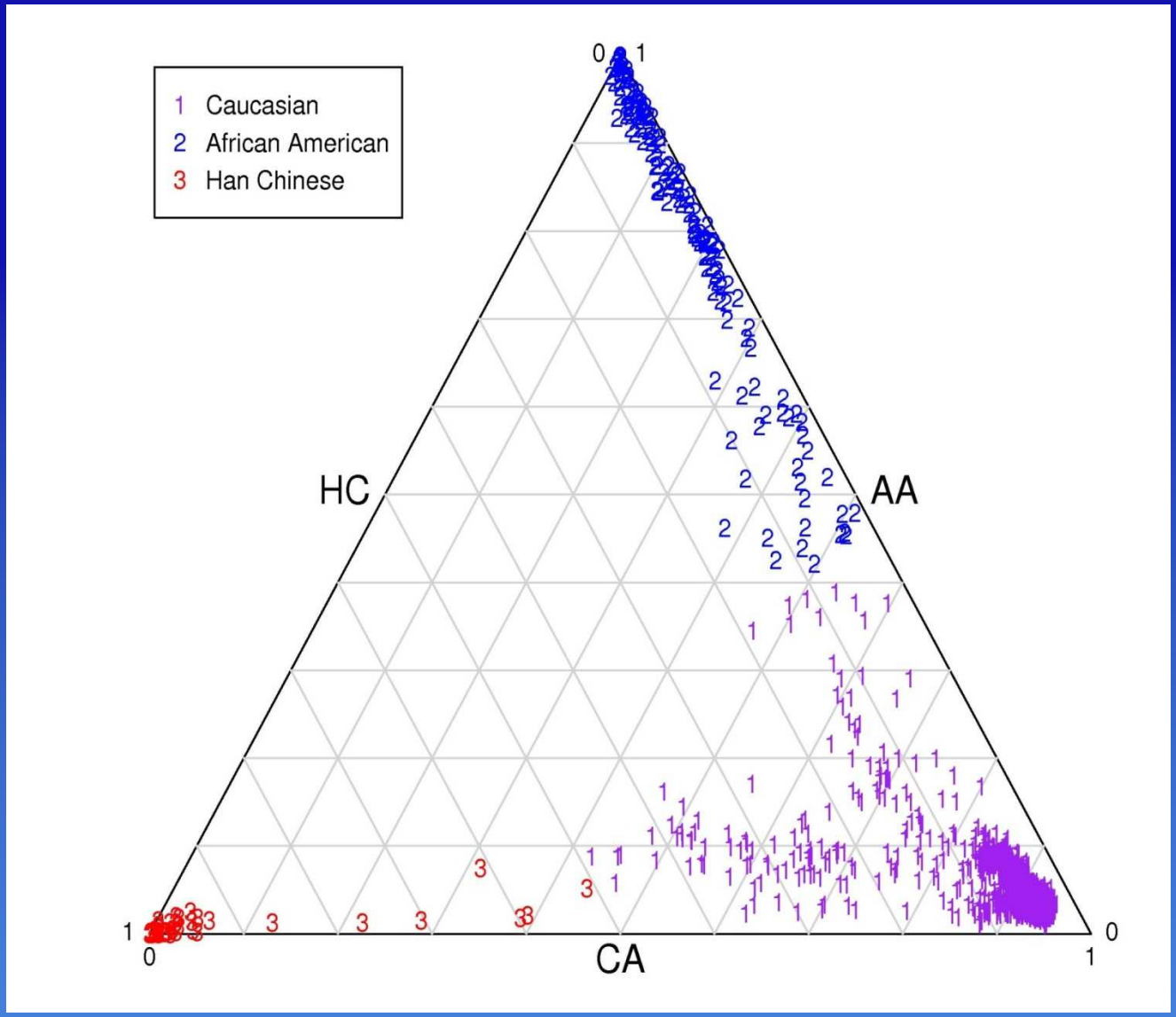
Final Race Classification, n=4657

Caucasian= 4449
Africans = 152
Han Chinese = 56



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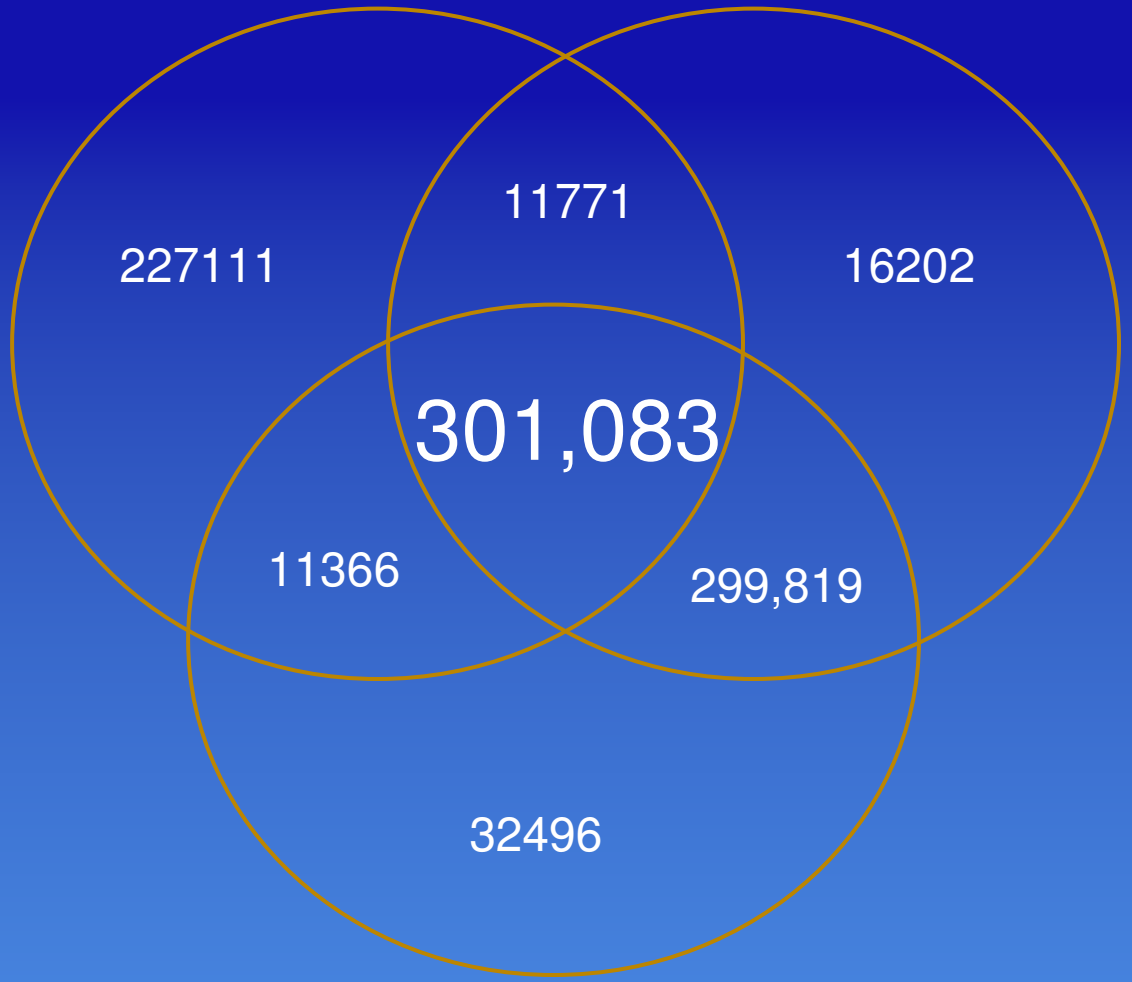
Genotyped SNPs in MA27 Studies n=899,848



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Imputed SNPs in MA27 Studies
MAF>0.01, R2>0.8

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Genotype QC Summary

Genotype QC on observed SNPs

# SNPs	excluded	Remaining	Reason
964193	1506+218+1199	961270	Chr Y, MT, and unplaced
961270	40631	920639	Failed SNPs
920639	250843	669796	MAF≤0.01
669796	0	669796	call rate ≤95%
669796	460	**669336**	HWE

Genotype QC after imputation

- After imputation, removed SNPs with MAF<0.01 and R^2<0.8 in all 3 cohorts
- Final number of SNPs for analysis from Imputation: **7,430,235**

Final number SNPs in analysis: 8,099,571

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Analysis

- 254 Events (breast recurrence)
- 4403 No Event
- Cox Proportional Hazard regression, adjusted for significant covariates.

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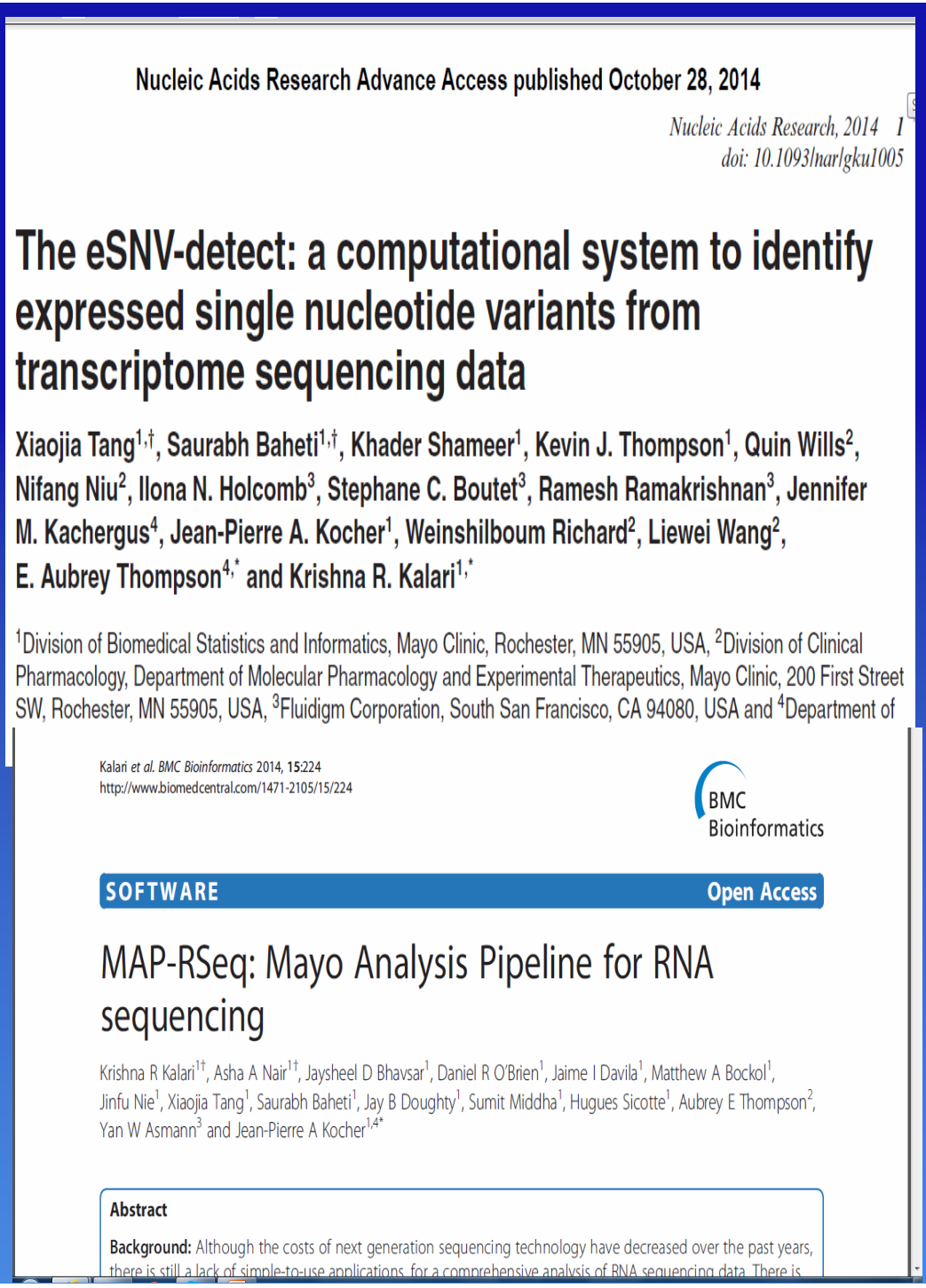
Future Directions

- GWAS complete
- Functional follow-up of Candidates
- The Cancer Genome Atlas data
- Breast Cancer Genome-Guided Therapy study (BEAUTY)



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The eSNV-detect: a computational system to identify expressed single nucleotide variants from transcriptome sequencing data

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MAP-RSeq: Mayo Analysis Pipeline for RNA sequencing

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Abstract

Background: Although the costs of next generation sequencing technology have decreased over the past years, there is still a lack of simple-to-use applications for a comprehensive analysis of RNA sequencing data. There is

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Thank you

Questions?