Pharmacogenetics/Pharmacogenomics (PGx) to Optimize Endocrine Therapy

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Clinical Observations with Endocrine Therapy

- **Variability** between patients in response, adverse events and end organ effects, e.g.,
  - Differences in disease outcomes
  - Striking differences in musculoskeletal events with AIs
  - DVTs, hot flashes, lipid effects with tamoxifen
- Genetic **variability** most certainly plays a role

Pharmacogenetics-Pharmacogenomics

- The study of the role of inheritance (i.e., the host genome) in individual variation in drug response phenotypes: i.e., disease response and end organ effects including adverse events
- Clinical goals
  - Better select responsive patients
  - Maximize drug efficacy
  - Minimize/avoid adverse drug reactions
- Integral to personalized medicine

Human Genome Project

Tenth Anniversary (Nature- Feb 10, 2011)

Evolution of Pharmacogenomic Translational Studies

- Study of one gene, one or a few SNPs at a time
- Study of PK and PD pathways and haplotypes
- Genome-wide association studies
- Whole genome DNA sequencing

With the massive advancement in technology, what have we delivered to our patients?

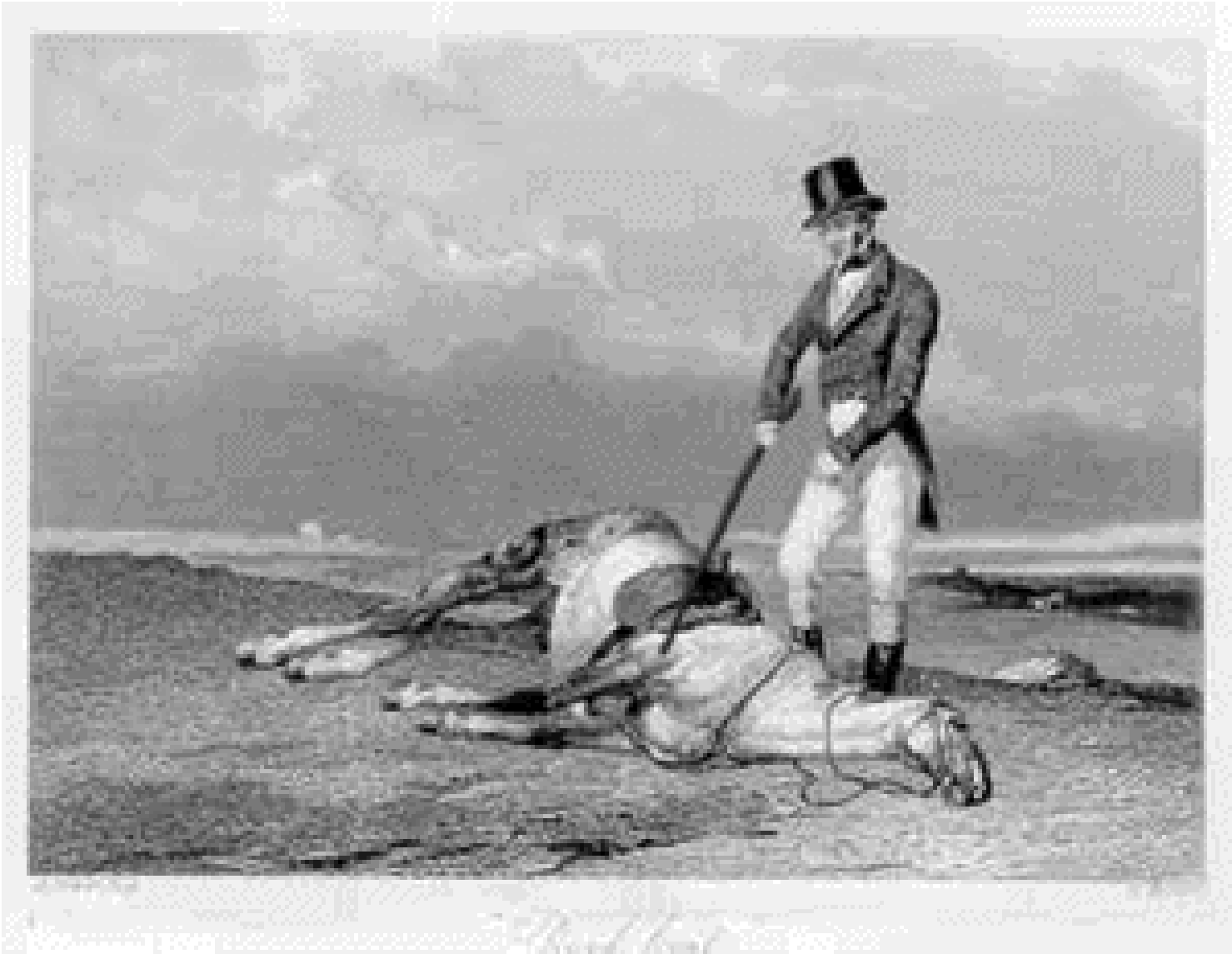
More specifically, what have we done to improve endocrine therapy for our patients?

Pharmacogenomic Translational Implementation

FDA Hearings-Relabeling-Warnings

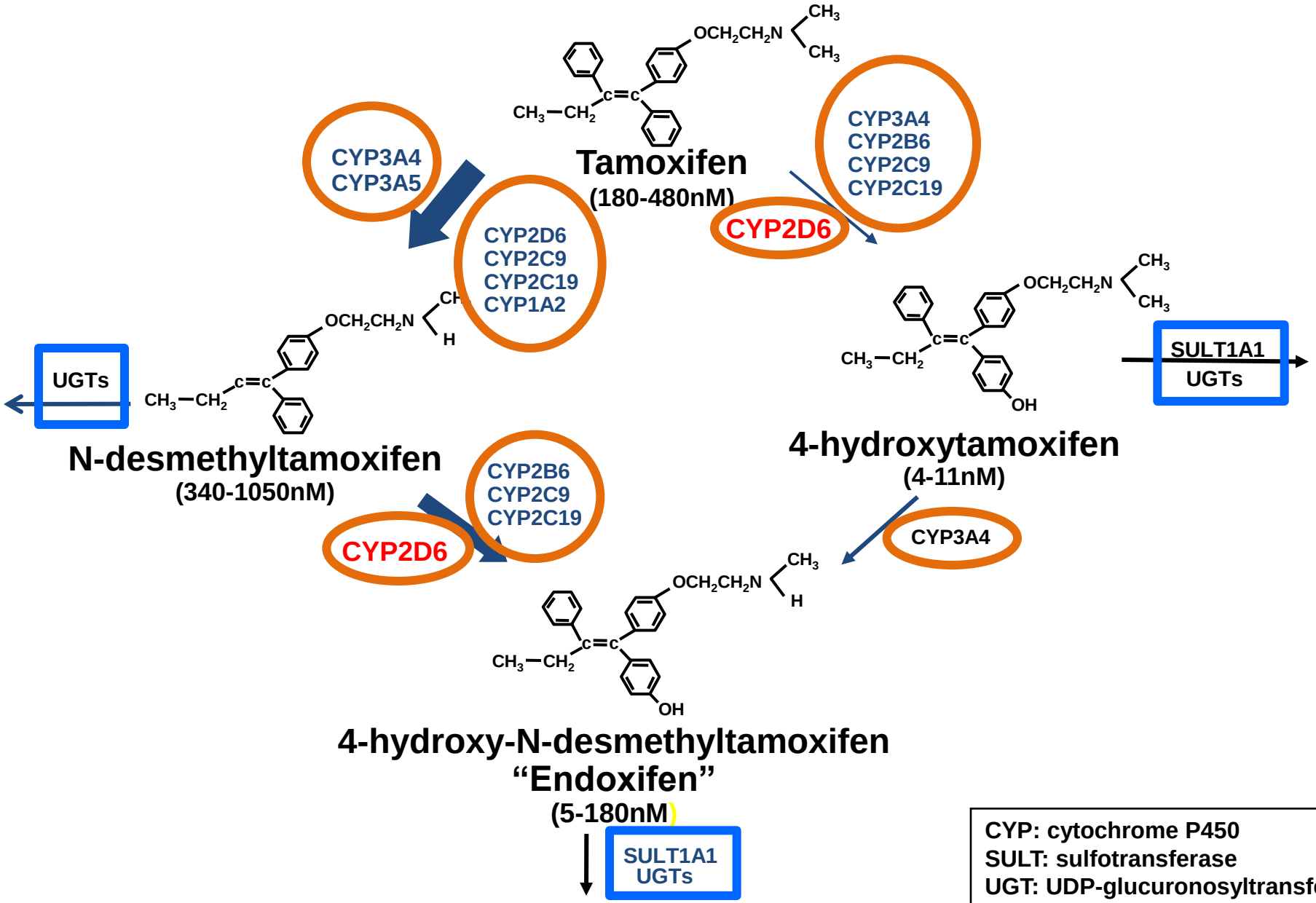
- Thiopurines – *TPMT*
- Irinotecan – *UGT1A1*
- Warfarin – *CYP2C9, CYP4F2* and *VKORC1*
- Tamoxifen – *CYP2D6*
- Codeine – *CYP2D6*
- Clopidrogel – *CYP2C19*

CYP2D6
Beating a Dead Horse?



Tamoxifen: Metabolic Pathway

Multiple Phase I and Phase II Enzymes are Involved



CYP: cytochrome P450
SULT: sulfotransferase
UGT: UDP-glucuronosyltransferase

Studies evaluating CYP2D6 and tamoxifen therapy have been retrospective and inconsistent

- First report showing worse outcome if poor metabolizer: Goetz, J Clin Oncol 2005

- Multiple subsequent studies: inconsistent results

- 2 subsequent studies: BIG 1-98 and ATAC showed no value of CYP2D6

Has this matter been “laid to rest”?

Consider several aspects: study design, genotyping, analysis plan

Selected Issues with Respect to PGx Studies of CYP2D6 (1)

- **Retrospective studies** must be carefully done with proper selection of patients, e.g., known ER+, treated with proper dose & duration, followed properly, adequate allele coverage, etc.
- reasonable standards have been proposed:

Use of Archived Specimens in Evaluation of Prognostic and Predictive Biomarkers

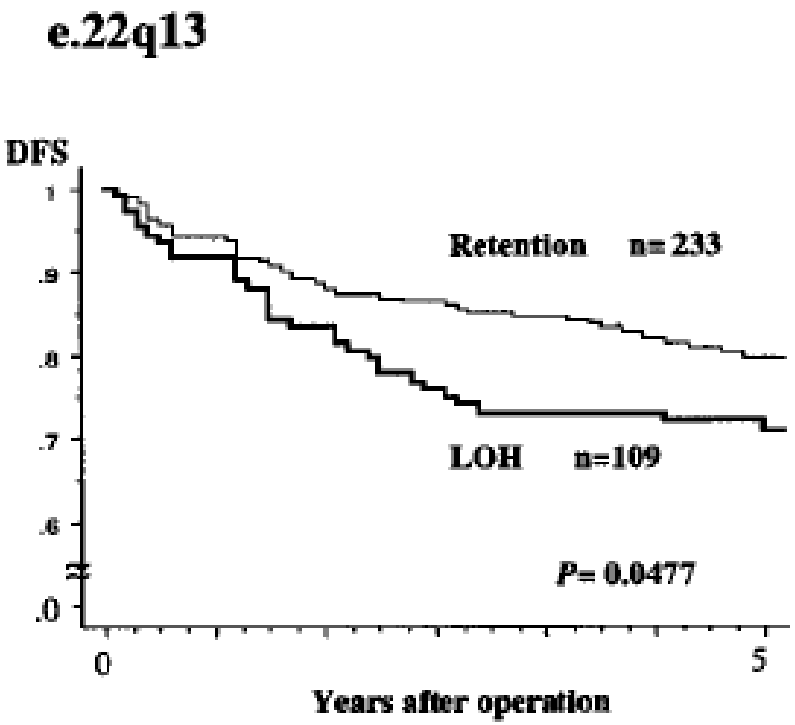
J Natl Cancer Inst 2009;101:1446–1452

Richard M. Simon, Soonmyung Paik, Daniel F. Hayes

- Adequate number of samples: suggest at least two-thirds be available
- Substantial data on analytical validity
- Complete development of analysis plan before assays
- Results must be validated in similarly designed studies

Selected Issues with Respect to PGx Studies of CYP2D6 (2)

- **Genotyping**: use of tumor can give misleading results, i.e., LOH at 22q13-location of CYP2D6 (32% loss in study of Hirano et al. Clin Cancer Res 2001;7:876-82)

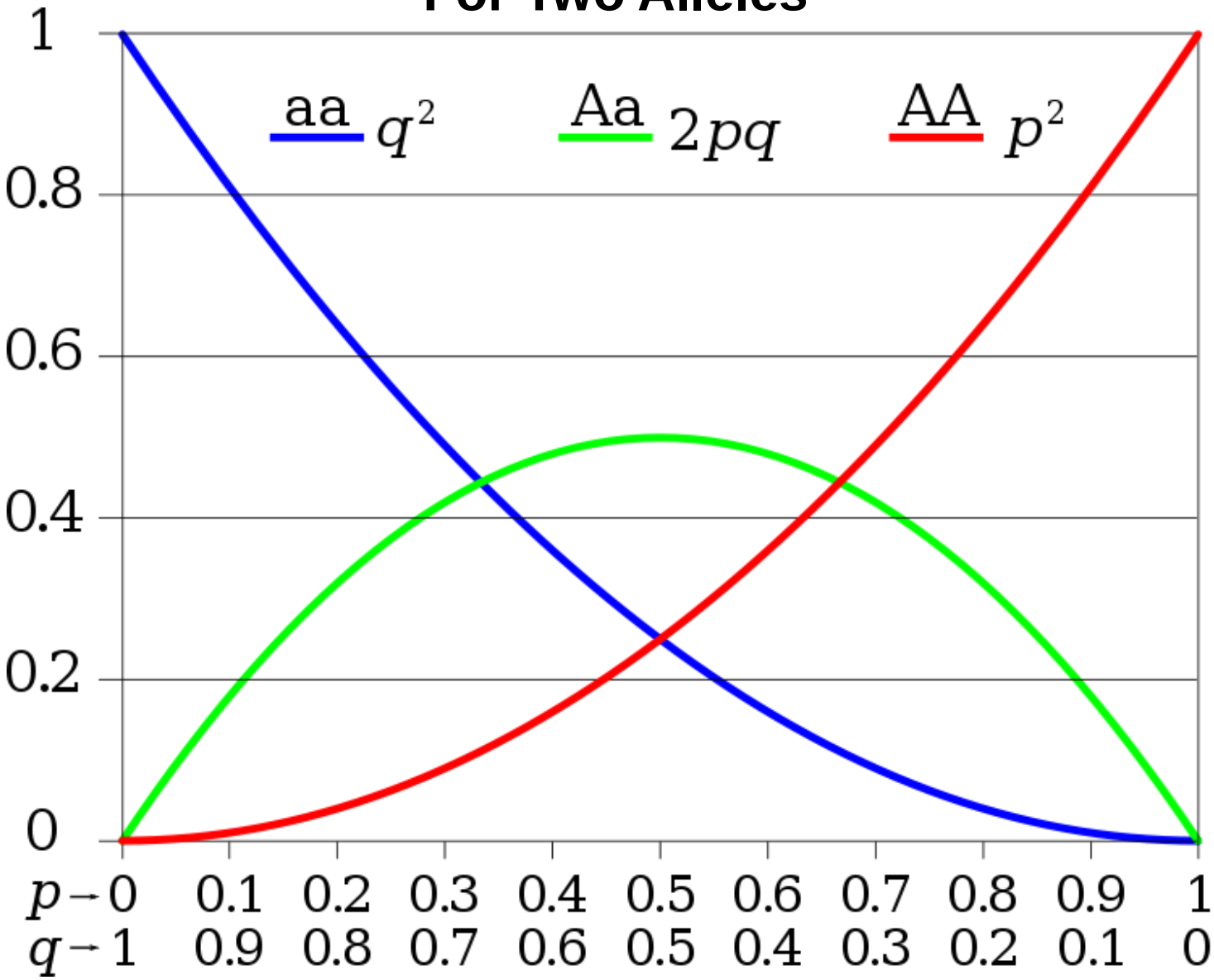


Selected Issues with Respect to PGx Studies of CYP2D6 (3)

- **Analysis plan**
  - Must start with quality control of multiple aspects of genotyping, e.g.,
    - Call rates for SNPs
    - HWE
  - Must adjust for multiple testing

Hardy-Weinberg Equilibrium

For Two Alleles



Wikipedia

CYP2D6 Genotype and Tamoxifen Response in Postmenopausal Women with Endocrine-Responsive Breast Cancer: The Breast International Group 1-98 Trial

Meredith M. Regan*, Brian Leyland-Jones*, Mark Bouzyk, Olivia Pagani, Weining Tang, Roswitha Kammler, Patrizia Dell'Orto, Maria Olivia Biasi, Beat Thürlimann, Maria B. Lyng, Henrik J. Ditzel, Patrick Neven, Marc Debled, Rudolf Maibach, Karen N. Price, Richard D. Gelber, Alan S. Coates, Aron Goldhirsch, James M. Rae, Giuseppe Viale; on behalf of the Breast International Group (BIG) 1-98 Collaborative Group

J Natl Cancer Inst 2012;104:1–11

Calculation for CYP2D6*4

A simple calculator to determine whether observed genotype frequencies are consistent with Hardy-Weinberg equilibrium

Genotypes	Observed #	Expected #
Homozygote reference:	2714	2520.8
Heterozygote:	784	1170.3
Homozygote variant:	329	135.8
	^Put your values here^	
Var allele freq:	0.19	

X^2 = 417.0213565

X^2 test *P* value = 0.000000 with 1 degree of freedom.

p=2.5E-92

1. If *P* < 0.05 - not consistent with HWE.

2. Not accurate if <5 individuals in any genotype group.

Michael H. Court (2005-2008)

URL: http:// tufts.edu/~mcourt01/Documents/Court%20lab%-%20HW%20calculator.xls

p=0.0000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000000025

Conclusions on CYP2D6

- The role of CYP2D6 in determining the value of tamoxifen therapy has not been resolved
- ITPC (International Tamoxifen Pharmacogenomics Consortium): 10 studies, report being prepared
- Several other studies to be reported
- PGx research is needed to properly determine the value of CYP2D6, especially important because tamoxifen remains the most widely employed endocrine therapy globally

Other PK & PD Considerations with Tamoxifen Efficacy

Data limited or non-existent

- Drug transporters
  - ABCB1 (adenosine triphosphate-binding cassette, MDR1)
  - ABCC2 (multidrug resistance-associated protein)
- Targets
  - ESR1
  - ESR2

Pharmacogenomic Genome-wide Association Studies in Endocrine Therapy of Breast Cancer

- Still in research realm
- Part of a process, i.e., the first step
- GWAS can identify a SNP that differs between cases and controls, but the SNP must be related to a gene and some function of that gene through functional genomic (laboratory) studies
- Our experience is that this process leads to identification of new biology
- Replication can be an issue when the GWAS is conducted using the largest studies (different from “Risk GWAS”)

Breast Cancer Prevention: Trials of Tamoxifen and Raloxifene

Tam vs Placebo	No. Patients
NSABP P-1	13,388
Royal Marsden Trial	2,471
Italian Trial	5,408
IBIS I	7,152
Raloxifene vs Placebo	
MORE	7,705
Tam vs Raloxifene (P-2)	19,471

32,859*

*59% of world's experience

GWAS of breast events and functional genomic studies in high-risk women receiving tamoxifen or raloxifene on NSABP P1 and P2 prevention trials

Nested matched case-control design involving 592 cases and 1171 controls

SPORE

PGRN

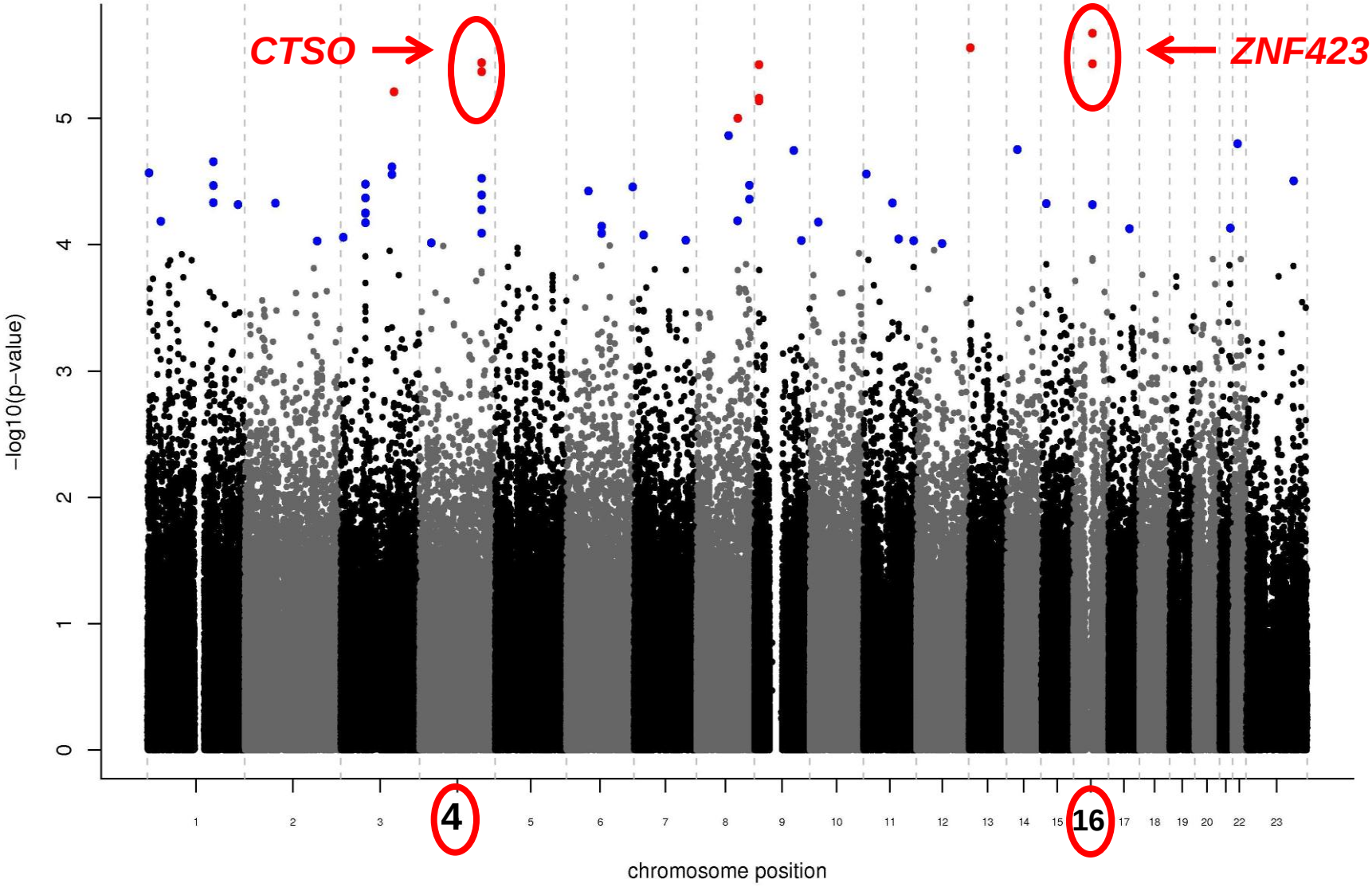
RIKEN

PGRN

NSABP®

NIH Global Alliance for Pharmacogenomics

Manhattan Plot of 547,356 SNPs*



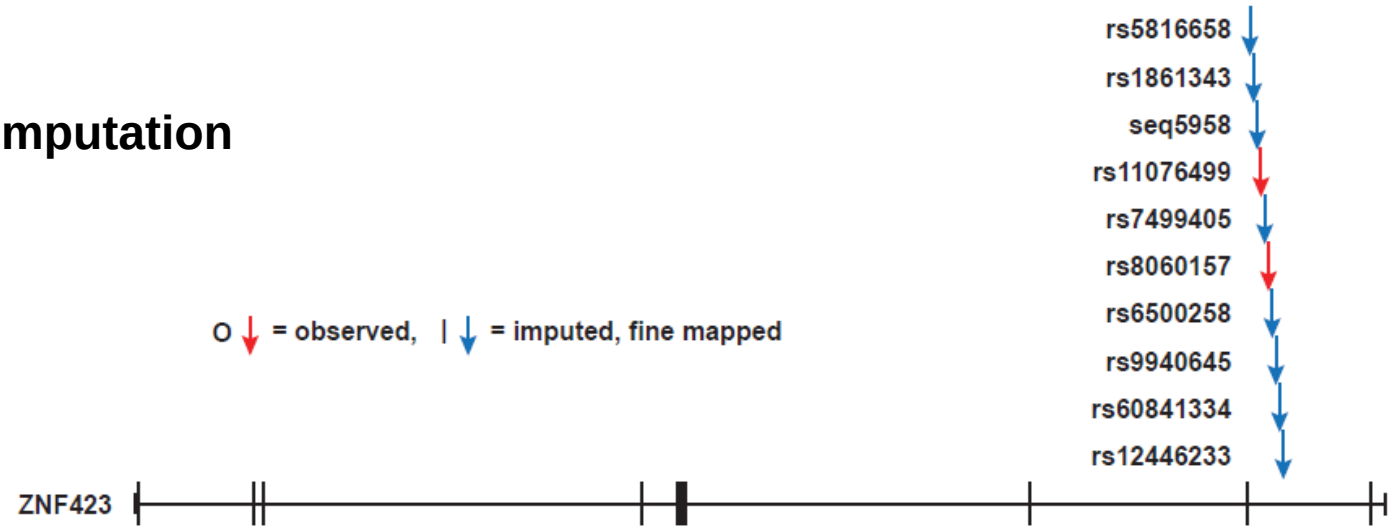
*Conditional Logistic Regression Analyses adjusted for 9 eigenvectors

Chr. 16: All 10 SNPs* with lowest P-values located in Exon 5 of *ZNF423*

SNP	Position (bp)	Minor Allele Frequency		Conditional Logistic Regression for Matched Design					Unconditional Logistic Regression
				Adjusted for 9 eigenvectors				Unadjusted	Adjusted for 9 eigenvectors
				OR	OR 95% CI				
		Cases	Controls		Lower	Upper	p-value	p-value	p-value
rs8060157	48387705	0.39	0.47	0.70	0.60	0.81	2.12E-06	2.86E-06	1.11E-06
rs6500258*	48388233	0.39	0.47	0.70	0.60	0.81	2.12E-06	2.86E-06	1.11E-06
rs9940645*	48389089	0.39	0.47	0.70	0.60	0.81	2.48E-06	3.17E-06	1.30E-06
rs7499405*	48386495	0.39	0.47	0.70	0.60	0.81	2.62E-06	3.36E-06	1.47E-06
rs60841334*	48389350	0.39	0.47	0.70	0.60	0.81	2.62E-06	3.36E-06	1.47E-06
seq5958*	48385458	0.39	0.47	0.70	0.60	0.81	2.82E-06	3.43E-06	1.56E-06
rs1861343*	48384669	0.39	0.47	0.71	0.61	0.82	3.70E-06	4.46E-06	1.87E-06
rs11076499	48385906	0.39	0.47	0.71	0.61	0.82	3.70E-06	4.46E-06	1.87E-06
rs5816658*	48384628	0.39	0.47	0.71	0.61	0.82	3.79E-06	4.94E-06	2.03E-06
rs12446233*	48389605	0.41	0.50	0.71	0.61	0.82	4.22E-06	5.37E-06	3.20E-06

* Imputed and genotyped

*** After imputation**

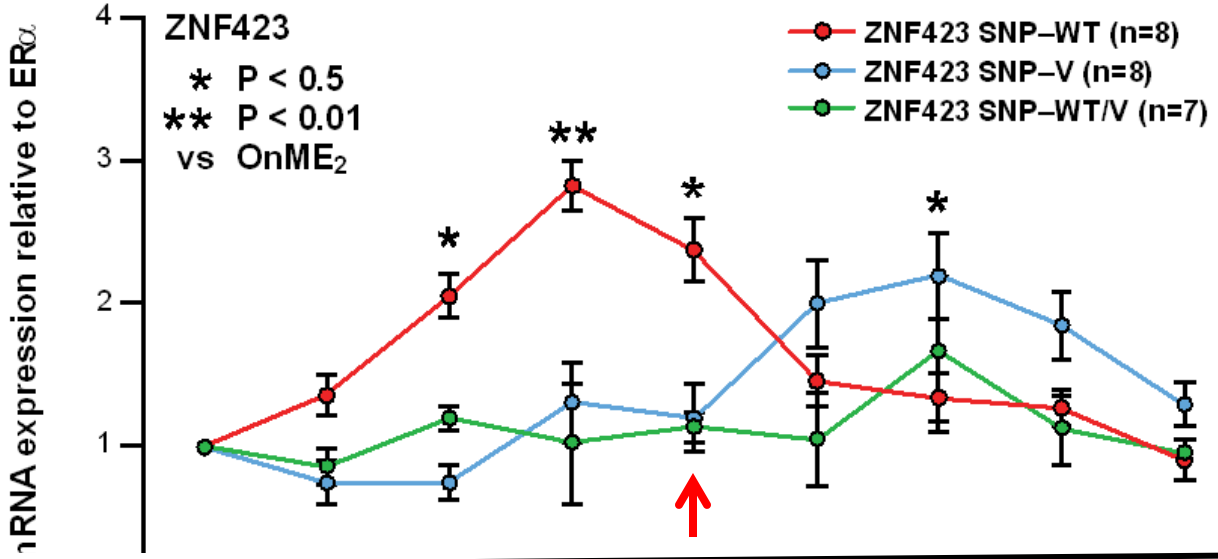


Summary of Functional Genomics Studies with *ZNF423* (variant protective, OR=0.7)

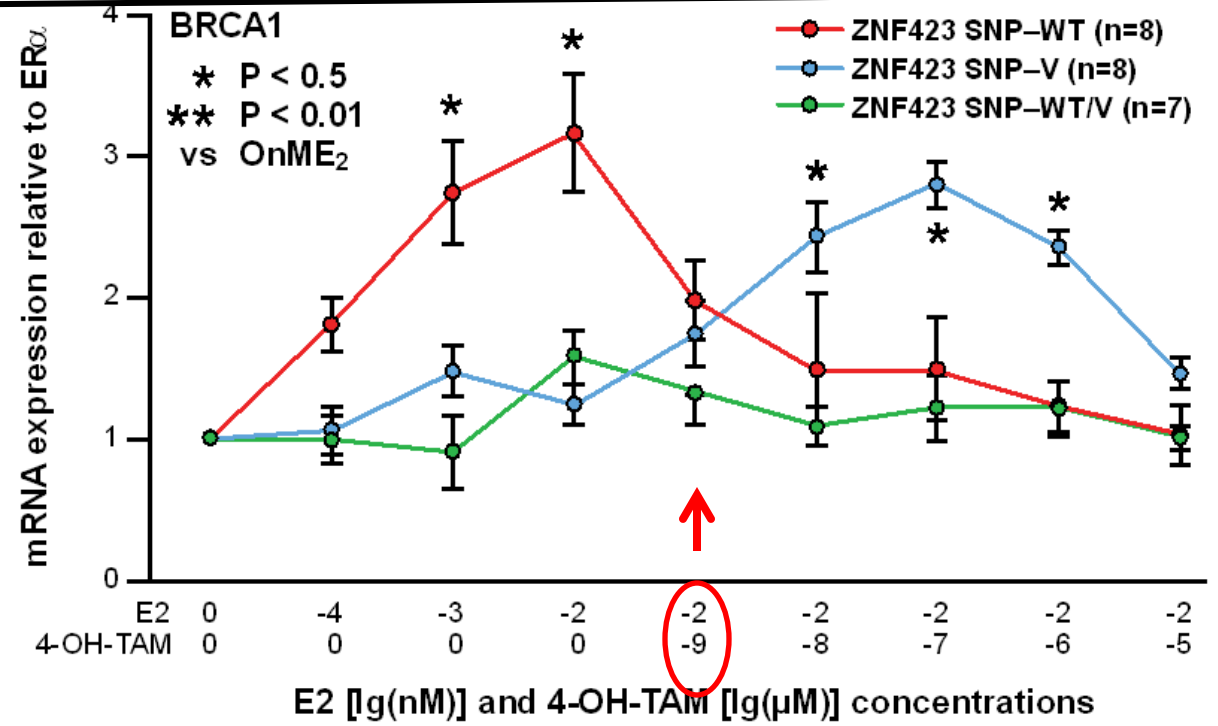
- ZNF423 expression is regulated by estradiol
- BRCA1 expression parallels that of ZNF423 in ERα stably transfected lymphoblastoid cell lines
- ZNF423 regulates *BRCA*1 5'-FR transcriptional activity
- ZNF423 binds the *BRCA1* 5'-FR (ChIP assay)
- Knockdown of ZNF423 and BRCA1 has the same effect on DNA damage phenotype
- *ZNF423* variant SNPs increase BRCA1 expression after ERα blockade with 4-OH-tamoxifen

ZNF423 Variant SNPs Increase BRCA1 Expression after ERα Blockade with 4-OH-Tamoxifen In Lymphoblastoid Cell Lines of Known ZNF423 Genotypes

ZNF423



BRCA1



Chr. 4: SNPs located near *CTSO** (variants deleterious, OR=1.4)
Summary of Functional Genomics Studies

- CTSO expression is regulated by estradiol
- BRCA1 expression also parallels that of CTSO (like ZNF423) in ERα stably transfected lymphoblastoid cell lines
- SNP rs6810983 variant disrupts an estrogen response element

*Cathepsin O

ZNF423 & CTSO SNP Joint Effect on Risk of Breast Cancer during SERM Therapy on P-1 and P-2

	CTSO [rs10030044 OR=1.4]		
ZNF423 [rs8060157 OR=0.7]	MM OR	Mm OR	mm OR
mm, OR	1.00 (9%)*	2.49 (21%)	4.71 (10%)
Mm, OR	1.86 (10%)	3.16 (24%)	3.55 (14%)
MM, OR	3.94 (3%)	3.88 (7%)	5.71 (4%)

*Percent of patients with genotype, OR: odds ratio for developing breast cancer in this high-risk population of patients

M = major allele m = minor allele

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38% patients have OR ≥3.55

NSABP P-1 AND P-2 GWAS Conclusions

- The NSABP GWAS discovered two genes “upstream” of BRCA1 that participate in E2-dependent BRCA1 expression
- These biomarkers may help make it possible to “individualize” SERM therapy
- These observations also provide a potential novel mechanism for individual variation in SERM response
- Clear implications for future clinical research

Aromatase Inhibitor Metabolism Phase I and Phase II Enzymes

- Anastrozole
  - CYP3A4
  - CYP3A5
  - CYP2C8
  - UGT1A4
  - UGT2B7
  - UGT1A3
- Exemestane
  - CYP3A4
  - UGTA10
  - UGT2B17
- Letrozole
  - CYP3A4
  - CYP2A6

A GWAS in Patients Experiencing Recurrence of Breast Cancer While Receiving AIs for Early Breast Cancer on NCIC CTG Trial MA.27 (anastrozole vs exemestane)

5221 of 6827 (76.4%) of North American patients will be genotyped

Study approved by NIH Global Alliance for Pharmacogenomics

James Ingle, Matthew Ellis, Paul Goss, Daniel Schaid, Judy-Anne Chapman, Lois Shepherd, Michiaki Kubo, Yusuke Nakamura, Richard Weinshilboum

Conclusions: Pharmacogenomics (PGx) in Endocrine Therapy

- PGx, at present, have not provided any established meaningful deliverables to our patients
- However, PGx studies have identified new biology that provides clear direction for further translational research with substantial potential for meaningful benefit to patients
- PGx is part of a process in scientific discovery
- Additional research, using robust cohorts or prospective trials, is needed