# Pharmacogenetics of Upper Aerodigestive Cancers: A Journey from hope to despair and back

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# Upper Aerodigestive Tract Cancers

- Head and Neck Cancer
- Esophageal Cancer
- Lung Cancer

- → “similar” epidemiological risk factors (smoking)
- → “similar” therapies (cisplatin), taxane)
- → “similar” markers (EGFR)

# Goals of Presentation

- Define Genetic Variation and Pharmacogenetics
- Present a historical perspective on development of this field in upper aerodigestive cancers
- Present opportunities for current and future research in this area

# Human Variation

- Each human person carries millions of normal variations in our DNA
  - Variations dictate everything from hair colour to shape of toenails
  - Common variations are called polymorphisms
  - We carry the same variations throughout life except when a mistake is made during cell division, where errors may lead to diseases such as cancer
  - Each parent passes ½ of our variations to our children

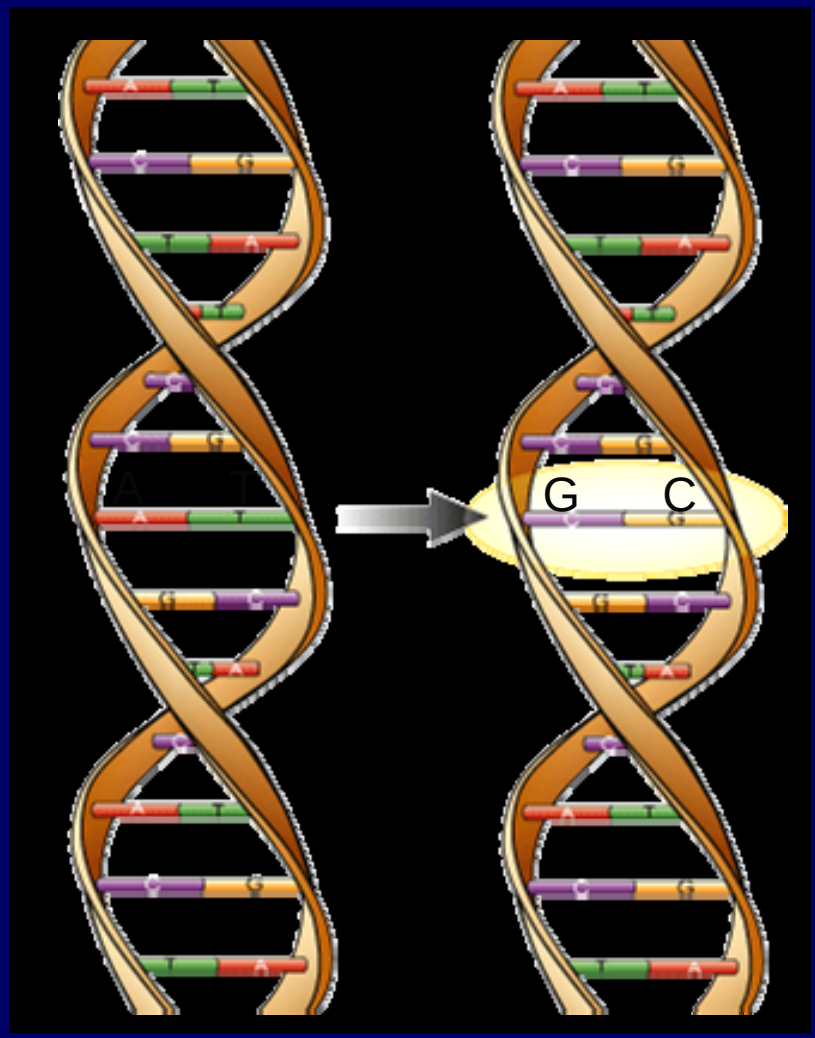
# Genetic Polymorphism

- Common variations in genetic code (>1% incidence in study population)
  - Otherwise called germline mutations
  - Inherited
  - Can be determined from a blood sample

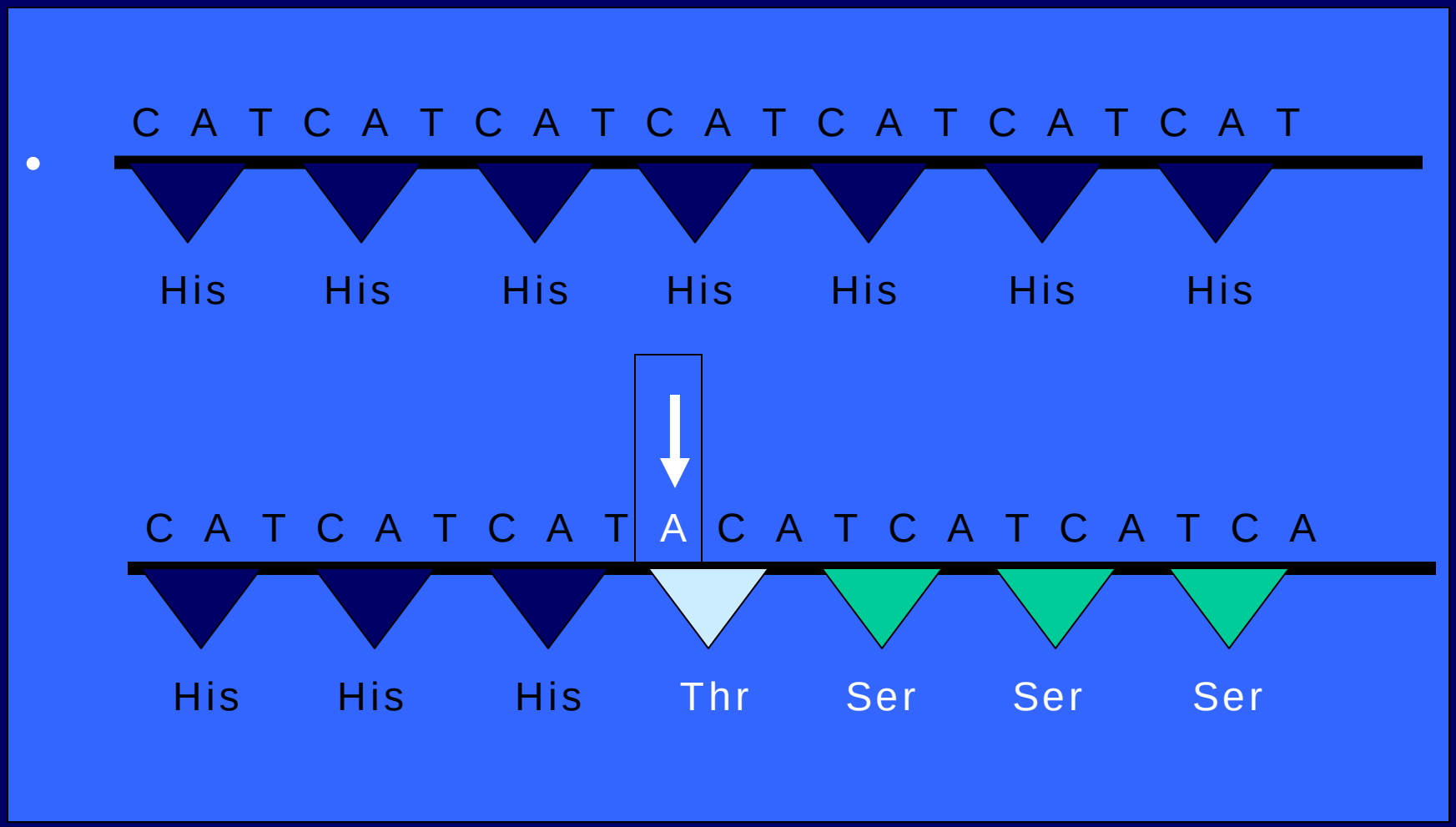
# Single Nucleotide Polymorphisms

- A Single substitution in the DNA sequence

- A → C
- A → T
- A → G

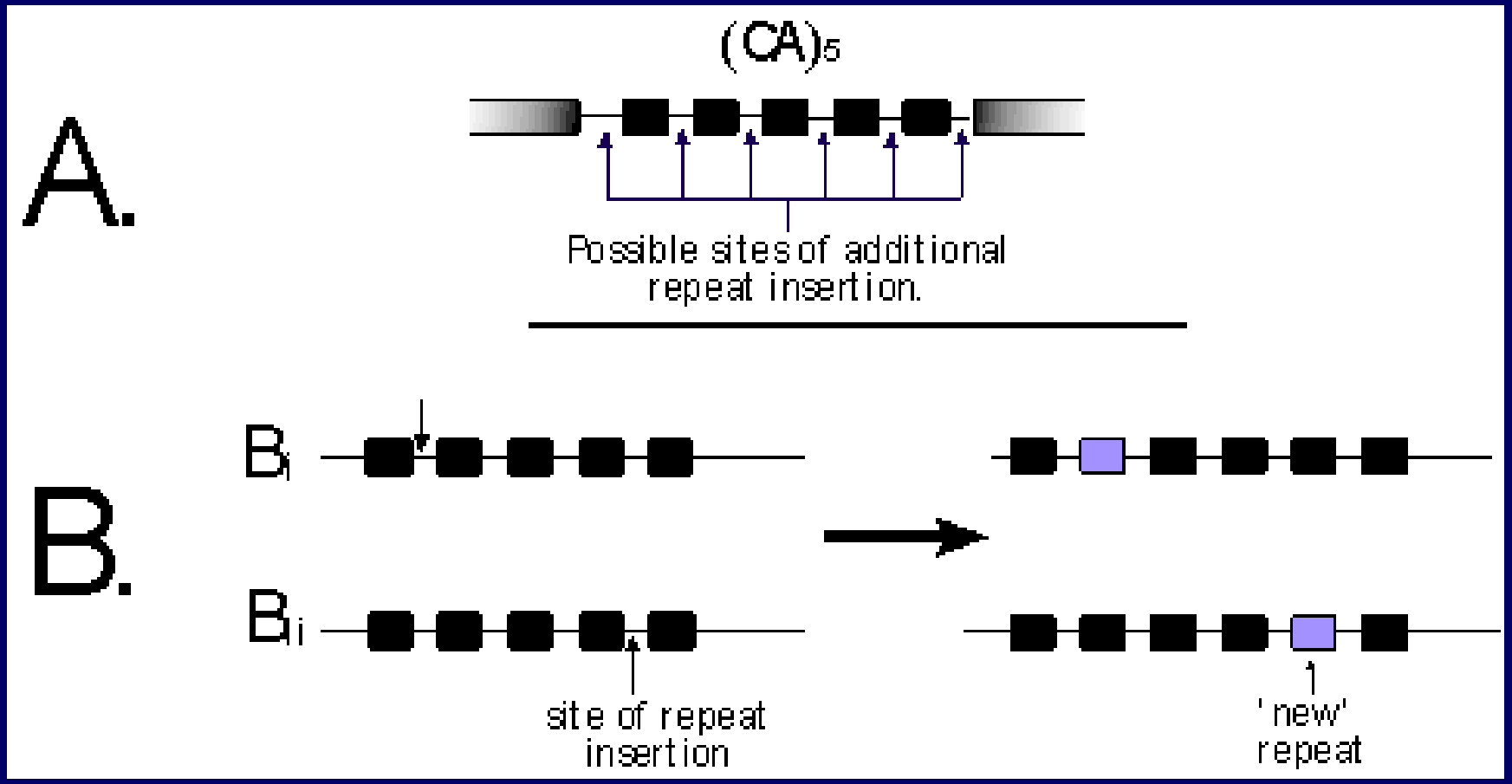


# Insertion (Deletion)



# VNTR (Variable Number Tandem Repeats)

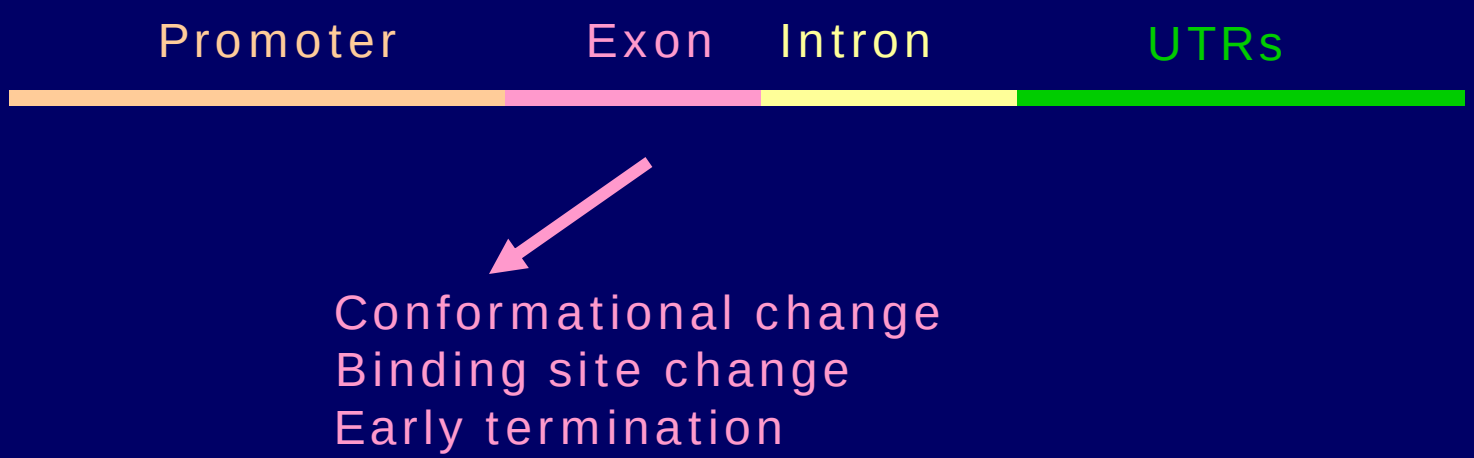
## Microsatellite



# Copy Number Variants

- A duplication or deletion involving > 1kb of DNA
  - Non-homologous end joining
  - Non allelic homologous recombination
  - Can affect expression levels, function

# Polymorphisms can alter function through multiple mechanisms



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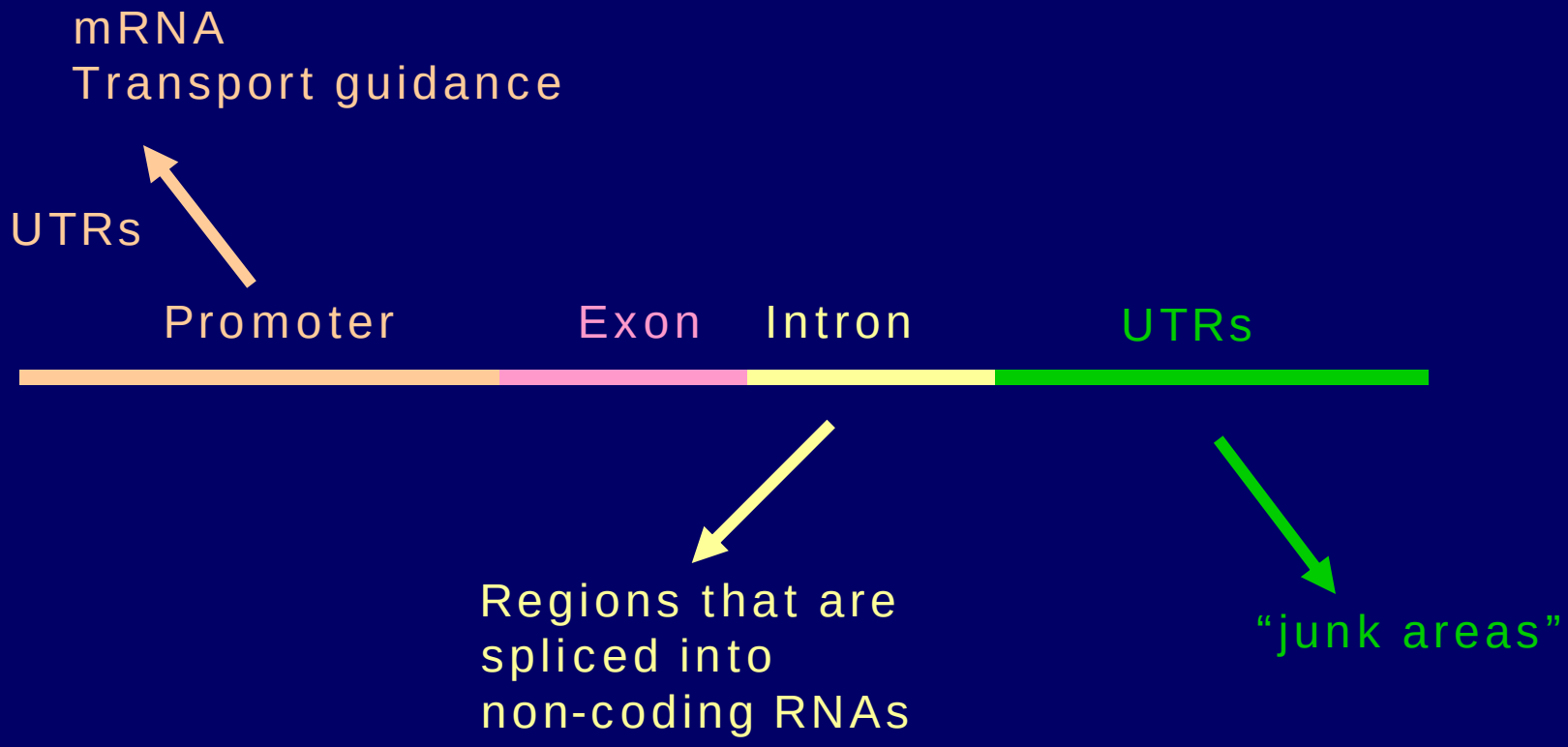


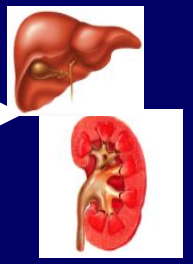
# Pharmacogenetics

- Pharmacogenetics: The study of how human variation (polymorphisms) affect our response to drugs

**PRO-DRUG**

**ACTIVE DRUG**

**INACTIVE DRUG**



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**Drug absorption**

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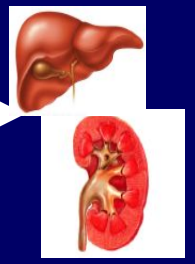

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- Pharmacogenetics: The study of how human variation (polymorphisms) affect our response to drugs

**Drug absorption**

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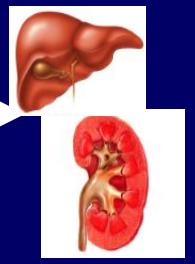
**Metabolic enzymes**

**ACTIVE DRUG**

**INACTIVE DRUG**



# Pharmacogenetics

- Pharmacogenetics: The study of how human variation (polymorphisms) affect our response to drugs

**Drug absorption**

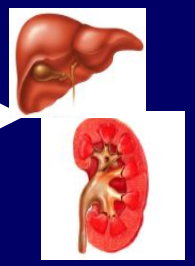
**PRO-DRUG**

**Metabolic enzymes**

**ACTIVE DRUG**

**Drug**
**- transporters**
**- binders**
**Body habitus**

**INACTIVE DRUG**



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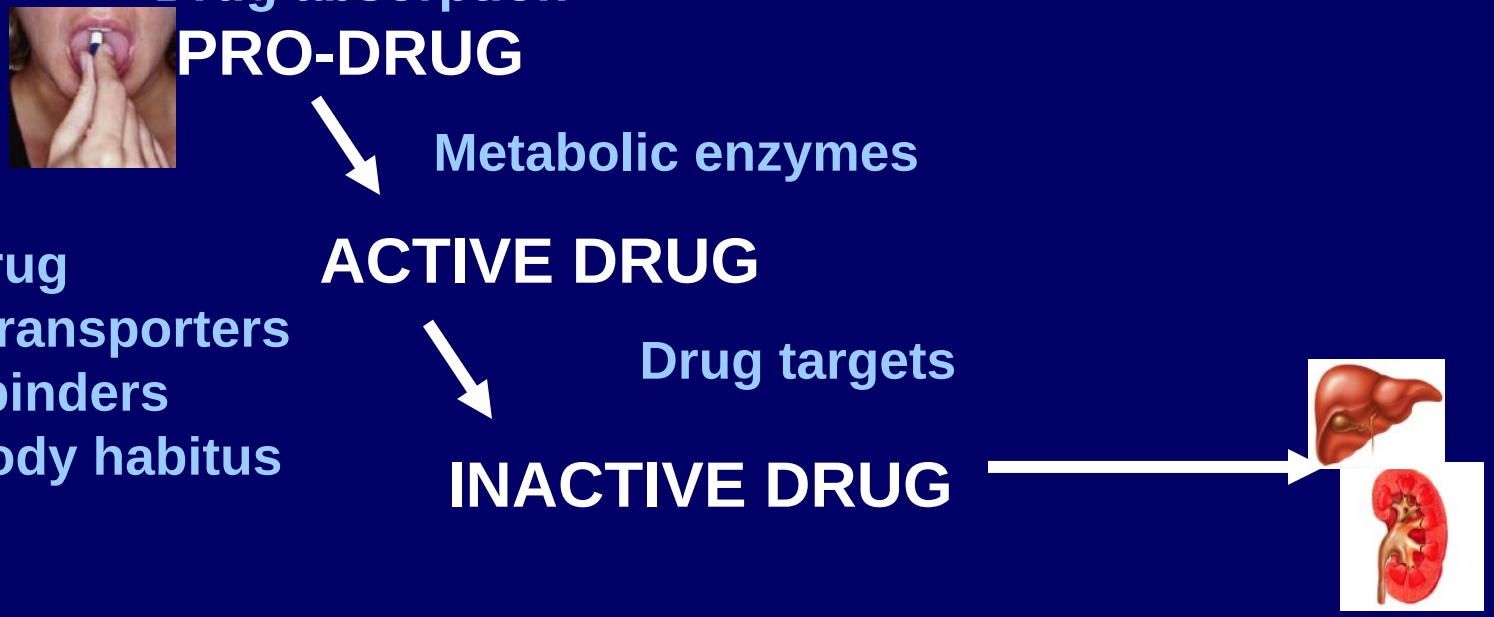


# Pharmacogenetics

- Pharmacogenetics: The study of how human variation (polymorphisms) affect our response to drugs

**Drug absorption**

**PRO-DRUG**

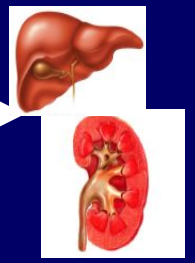
**Metabolic enzymes**

**ACTIVE DRUG**

**Drug**
**- transporters**
**- binders**
**Body habitus**

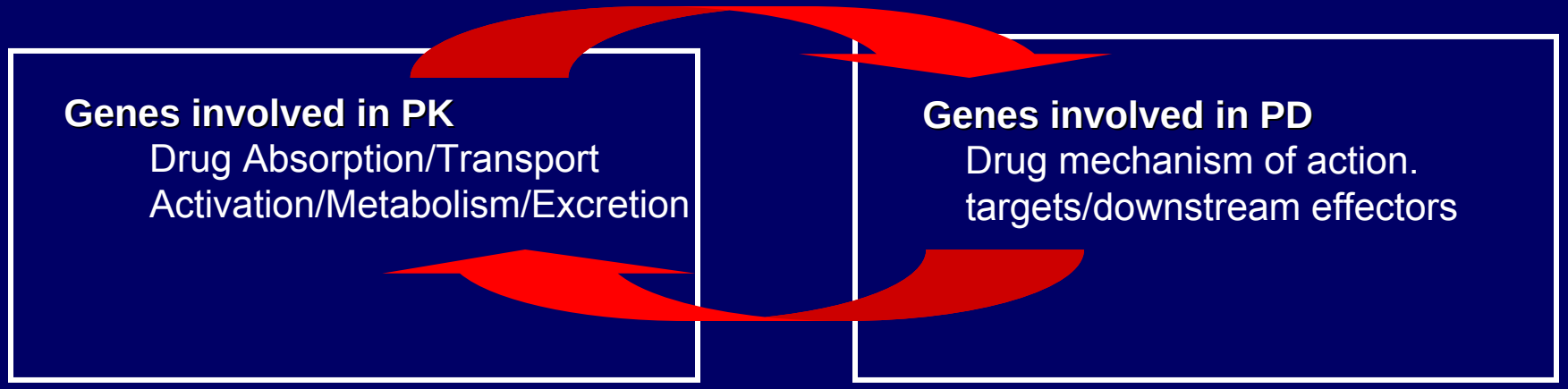
**Drug targets**

**INACTIVE DRUG**



**Drug excretion**

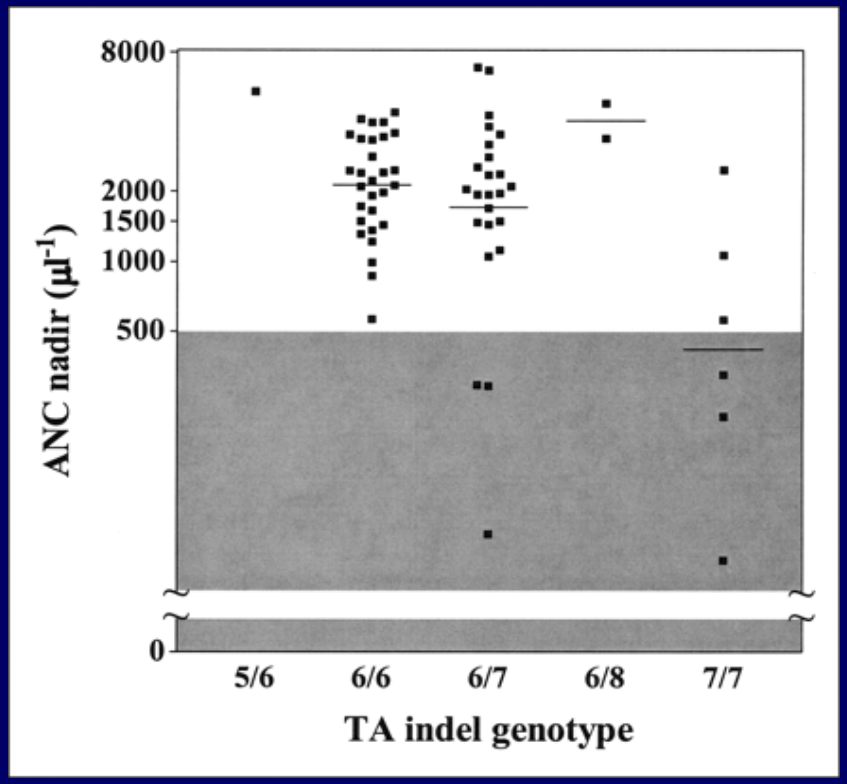
# History of "Successes" in Pharmacogenetics




**Hematology/OncologyDrugs with FDA label modifications**

| *Drug* | *Genetic Variation* | *Involved in:* | *Outcome* |
|---|---|---|---|
| 6MP and AZA | *TPMT* | PK | Toxicity |
| Irinotecan | *UGT1A1* | PK | Toxicity |
| Warfarin | *CYP2C9 & VKORC1* | PK and PD | Toxicity |
| Tamoxifen | *CYP2D6* | PK | Efficacy |

# UGT1A1 gene and irinotecan: TA indel polymorphism is associated with ANC nadir

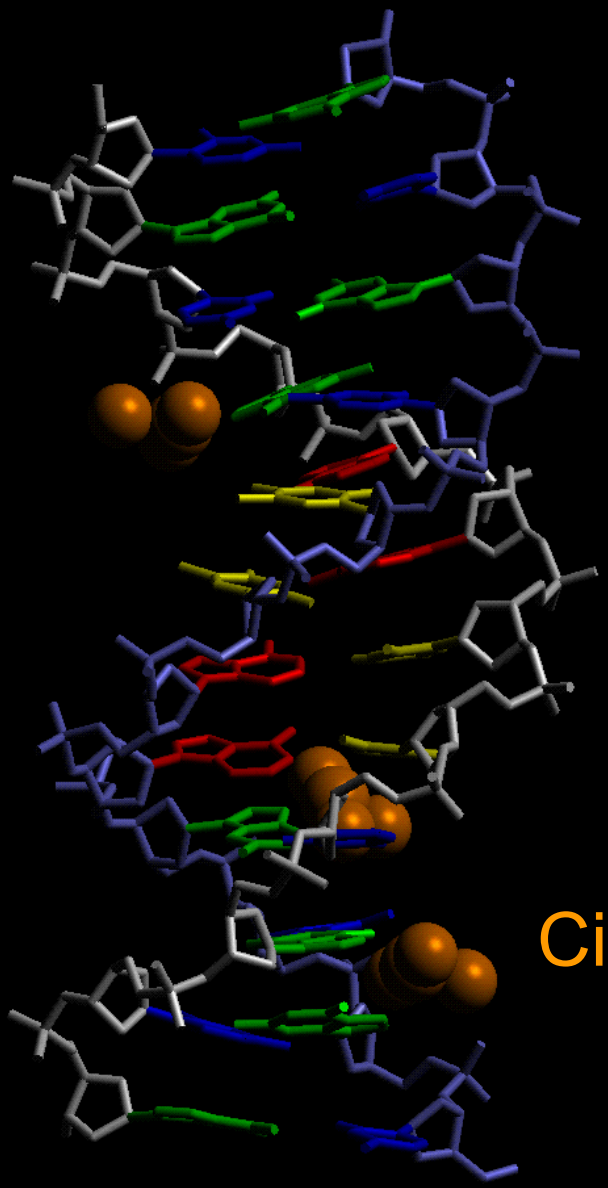



Correlation between absolute neutrophil count (ANC) nadir (log scale) and TA indel genotype

# 1. Candidate polymorphism approach

- Data supporting association with disease, outcome, or function
  - Biologic (genotype-phenotype, *in vivo* studies)
  - *In silico* “predictive function”
  - Evolutionary
  - Epidemiologic

# Platinum-DNA adduct formation



Cisplatin

# *XPD* and *XRCC1* polymorphisms

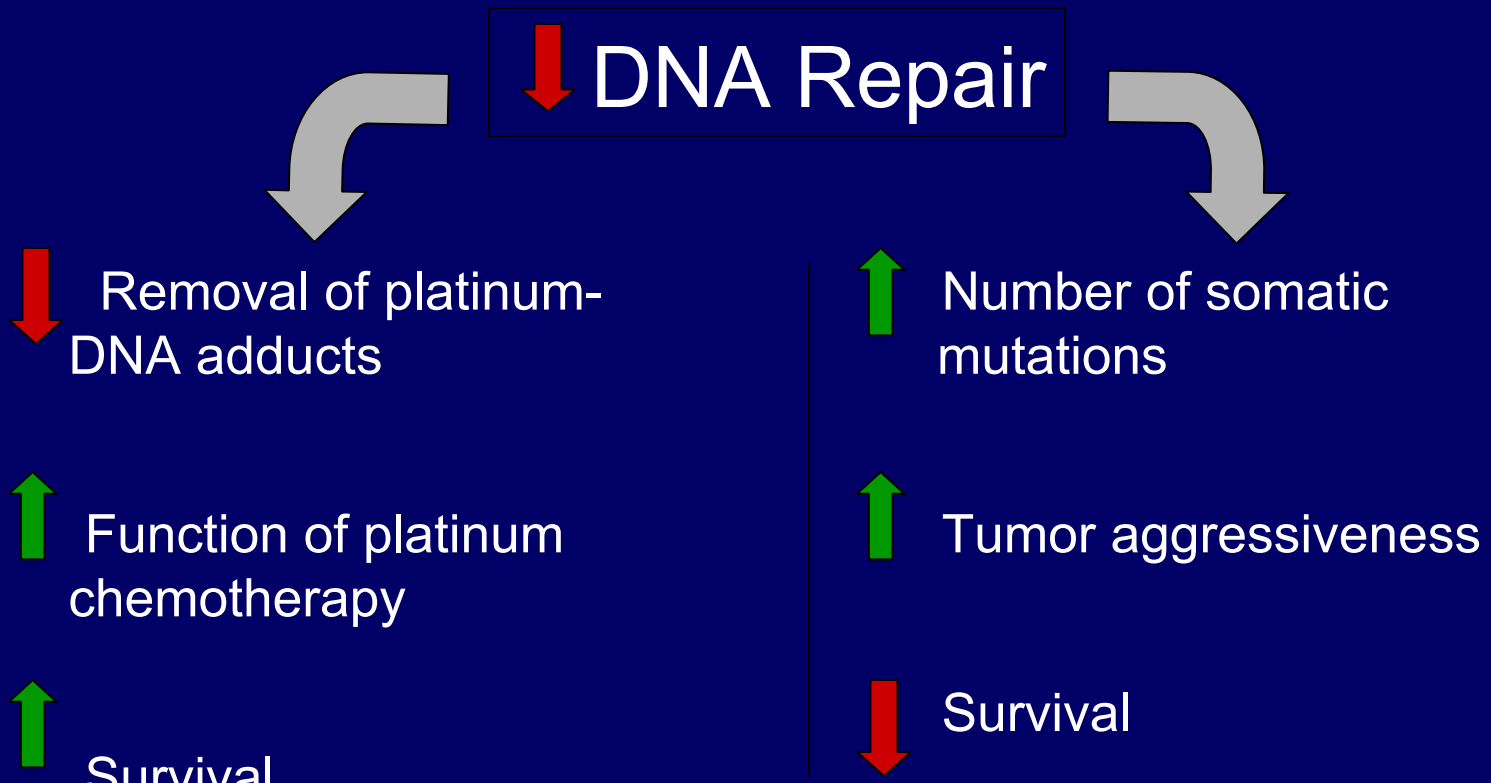
- Differential activity
- Case-control studies of lung cancer *risk*

→ *Can these polymorphisms explain differences in outcome after platinum treatment in NSCLC patients?*

# Hypothesis

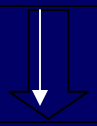
- Variant genotypes of the DNA repair genes, *XPD* and *XRCC1*, affect survival in advanced NSCLC patients treated with platinum-based regimens

# Effect of DNA repair on outcome



# Patient selection

251 patients with histologically-proven advanced NSCLC, 5+ years follow-up and available medical records



112 patients treated with platinum agents at MGH Cancer Center



103 patients with complete genotype data for *XPD* and *XRCC1*

# Genotyping

- DNA from whole blood
- Genotyping by PCR-RFLP
  - *XPD/ERCC2 (Asp312Asn)*
  - *XRCC1 (Arg399Gln)*

# Clinical Outcome

- Overall survival
- Dates of death confirmed through
  - SSDI
  - Outpatient/inpatient records
  - MGH tumor registry
- Patients not deceased were censored at
  - Last date of clinic follow-up or
  - Last date known alive

# Patient Characteristics

| Demographics | | N |
|---|---|---|
| Total | | 103 |
| Stage | IIIA<br>IIIB<br>IV | 26 (25%)<br>30 (29%)<br>47 (46%) |
| Gender | Male<br>Female | 53 (51%)<br>50 (49%) |
| No. of events | | 86 |
| Median age | | 58 (32-77) |

Gurubhagavatula et al, JCO 2005

# Median Survival Times

| | | n | MST (months) |
|---|---|---|---|
| Total | | 103 | 14.9 |
| By Stage | IIIA | 26 | 28.6 |
| | IIIB | 30 | 16.0 |
| | IV | 47 | 9.3 |
| Median f/u time | | | 63.9 months |

**Stage was not associated with any genotypes**

Gurubhagavatula et al, JCO 2005

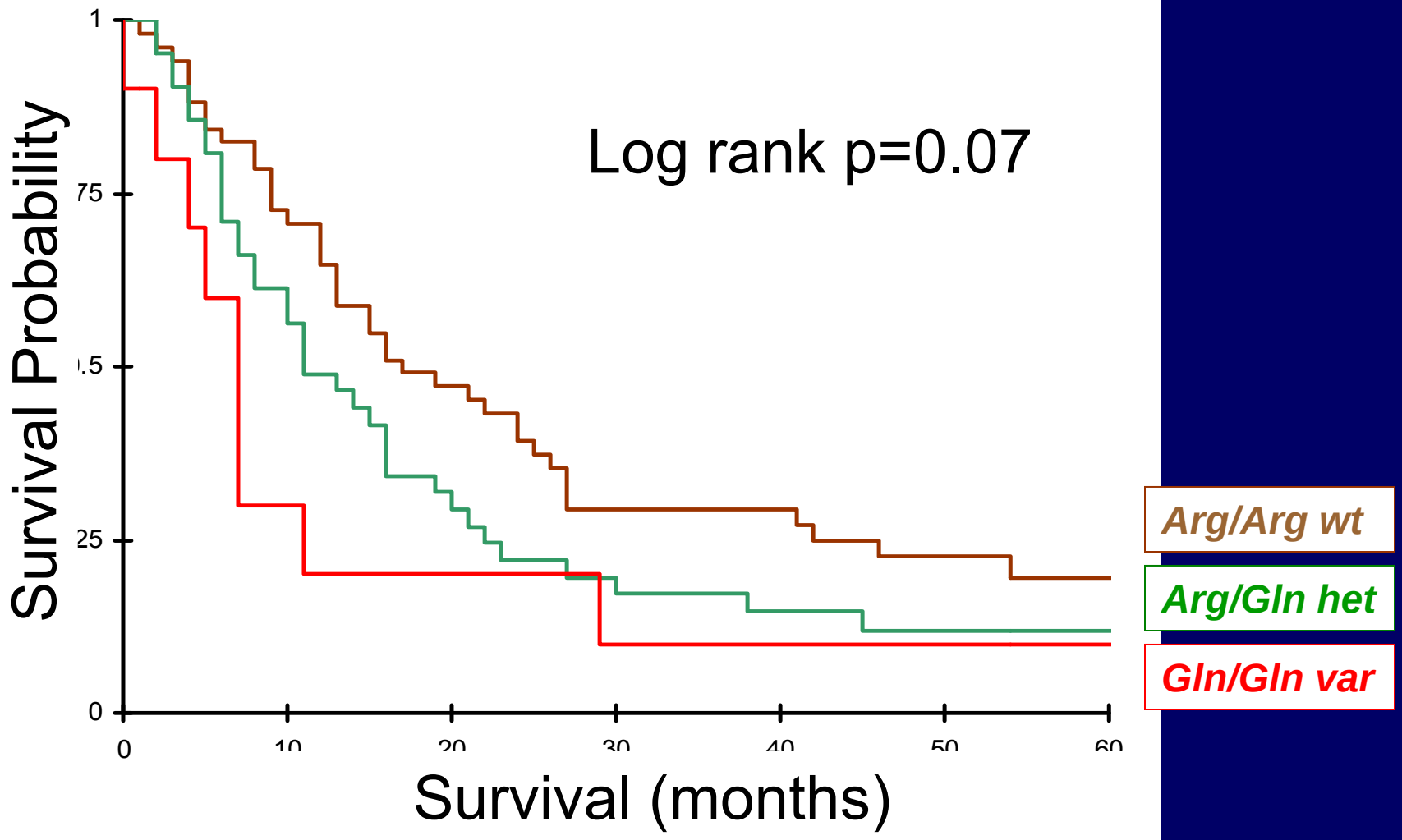
# *XRCC1* variant genotypes are associated with poorer survival

| Genetic polymorphism | | n | MST (mos) | Logrank test | Hazard Ratio (95% CI)* |
|---|---|---|---|---|---|
| ***XRCC1 Arg399Gln*** | *Arg/Arg*** (Wildtype) | 51 | 17.3 | **p=0.07** | 1.0 (reference) |
| | *Arg/Gln* (Hetero) | 42 | 11.4 | | 1.45 (1.03-2.05) |
| | *Gln/Gln* (Variant) | 10 | 7.7 | | 2.11 (1.49-2.98) |

*by Cox proportional hazards model adjusted for stage and PS
**homozygous wildtype

Gurubhagavatula et al, JCO 2005

## *XRCC1* Genotypes and Overall Survival




Gurubhagavatula et al, JCO 2005

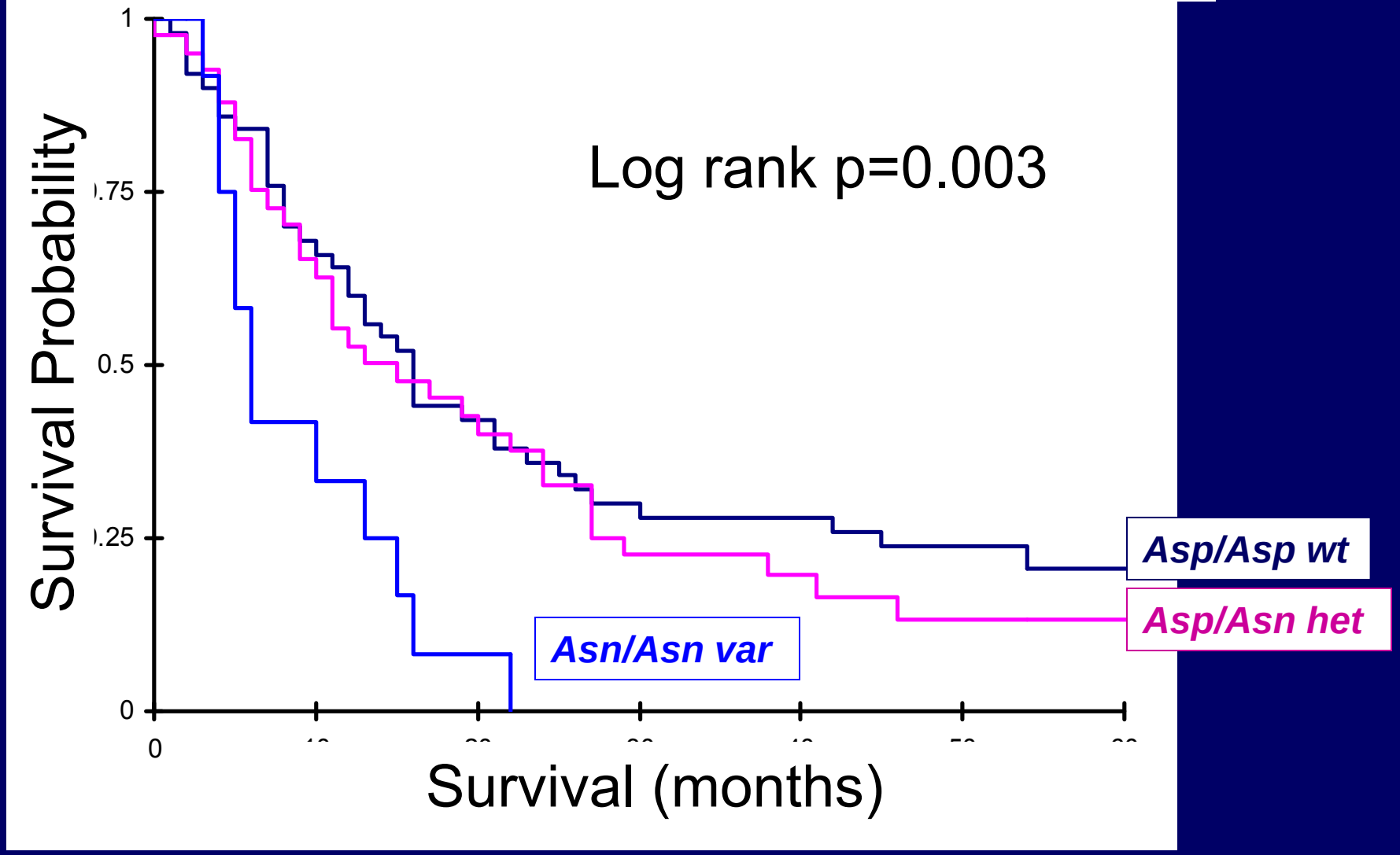
# *XPD* variant genotypes are associated with poorer survival

| Genetic polymorphism | | n | MST (mos) | Logrank test | Hazard Ratio (95% CI)* |
|---|---|---|---|---|---|
| ***XPD Asp312Asn*** | *Asp/Asp*** (Wildtype) | 50 | 16.3 | **p=0.003** | 1.0 (reference) |
| | *Asp/Asn* (Hetero) | 41 | 15.2 | | 1.36 (0.97-1.90) |
| | *Asn/Asn* (Variant) | 12 | 6.6 | | 1.84 (1.31-2.58) |

*by Cox proportional hazards model adjusted for stage and PS
**homozygous wildtype

Gurubhagavatula et al, JCO 2005

## *XPD* Genotypes and Overall Survival




Gurubhagavatula et al, JCO 2005

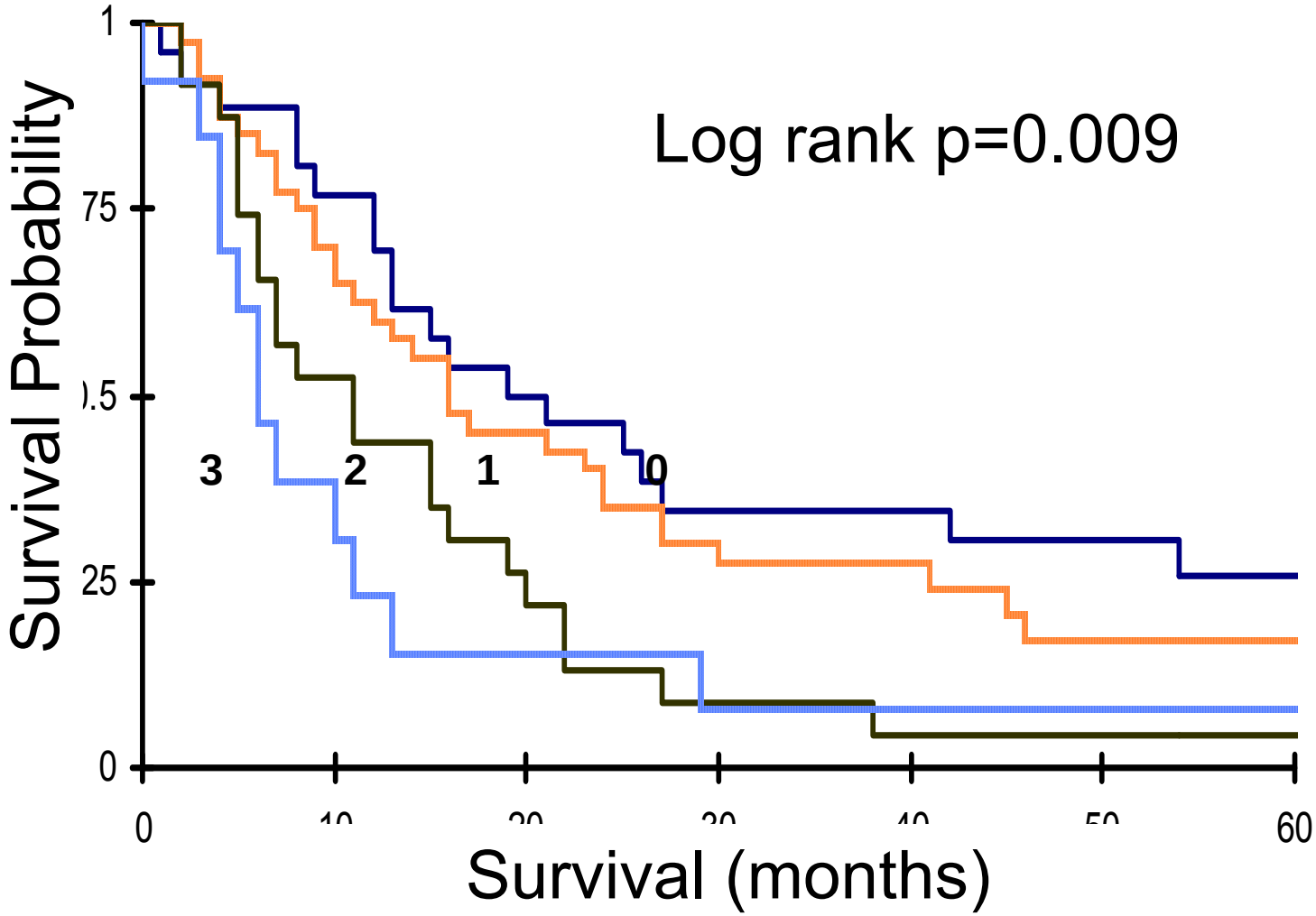
# The combination of variant genotypes is associated with poorer survival

| Genetic polymorphism | | n | MST (mos) | Logrank test | Hazard Ratio (95% CI)* |
|---|---|---|---|---|---|
| **Combined** | 0 variants** | 26 | 20.4 | **p=0.009** | 1.0 (reference) |
| | 1 variant allele | 40 | 16.6 | | 1.41 (1.11-1.80) |
| | 2 variant alleles | 24 | 11.0 | | 1.99 (1.56-2.53) |
| | 3 variant alleles | 13 | 6.8 | | 2.80 (2.20-3.57) |

*by Cox proportional hazards model adjusted for stage and PS
**double homozygous wildtype

Gurubhagavatula et al, JCO 2005

## Number of *XPD/XRCC1* Variant Alleles and OS

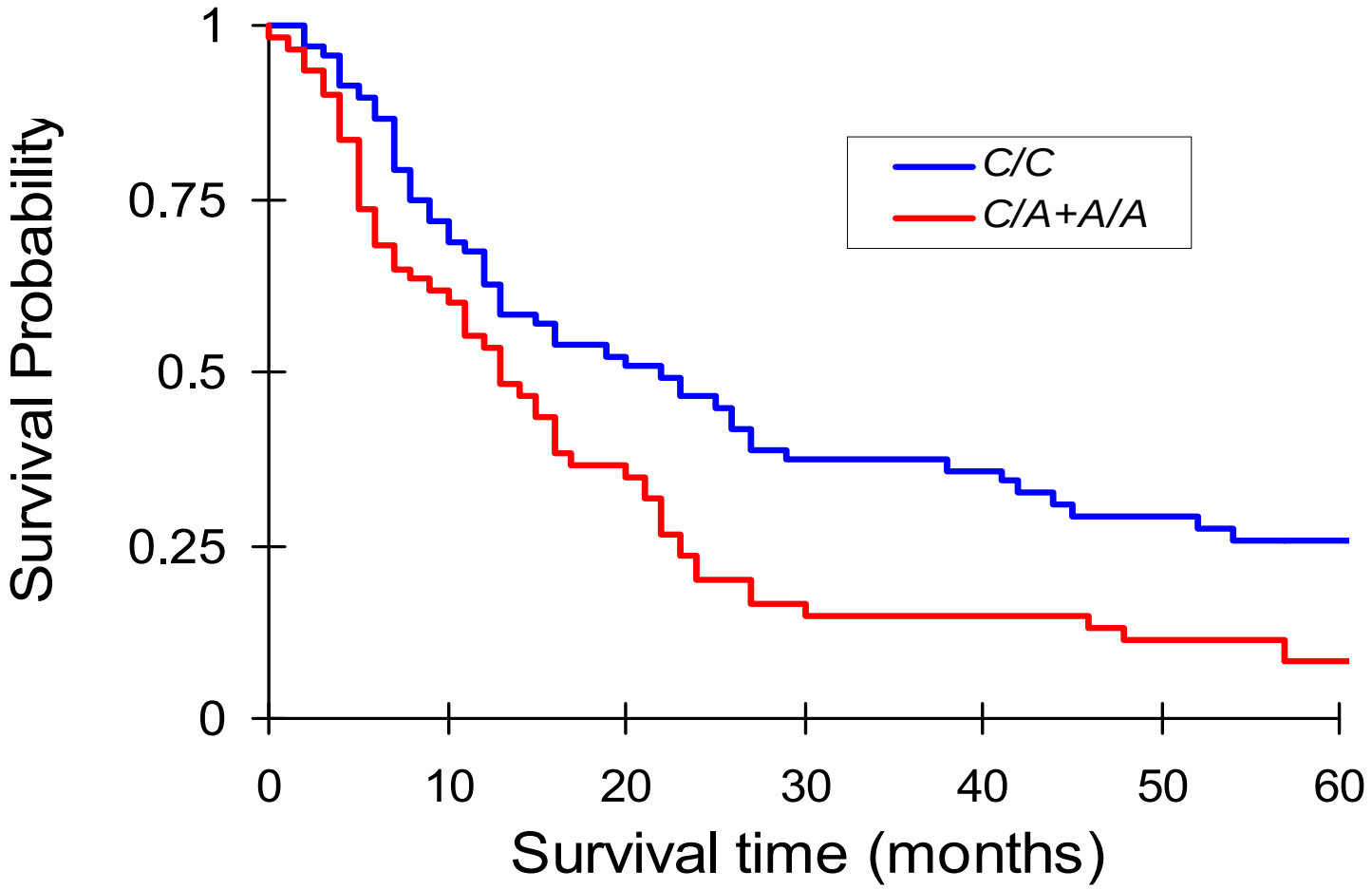



Gurubhagavatula et al, JCO 2005

# Summary

- Evaluation of DNA repair gene polymorphisms is feasible
- *XPD* and *XRCC1* variant genotypes, both alone and in combination, are associated with decreased overall survival in platinum-treated NSCLC patients

- …then information surfaced on the importance of ERCC1 in cisplatin-related DNA repair




**Kaplan-Meier curves of the *ERCC1 C8092A* polymorphism (P=0.006, by logrank test)**

Zhou et al, CCR 2005

# DNA Repair Polymorphism and Grade III/IV Gastrointestinal (GI) Toxicity

- **147 NSCLC patients treated first-line with combined chest radiation and platinum-based chemotherapy**
- **93% were PS ECOG 0/1**
- **Stage**
  - **6% were stage I and II, 46% were stage IIIA, 39% were stage IIIB, and 9% were stage IV**
- **Treatment**
  - **42% received cisplatin, 58% received carboplatin**
- **Thirty-one (21%) patients experienced Grade III/IV GI toxicity (nausea, n=10; vomiting, n=4; esophagitis, n=20)**

ASCO abstract, 2004

Zhou et al, CCR 2005

## *ERCC1* polymorphism and GI toxicity

| | ***ERCC1* C8092A** | **Total n** | **Grade III/IV GI toxicity: n (%)** | **Adjusted OR (95% CI)** |
|---|---|---|---|---|
| **All stages** | *C/C*<br>*C/A or A/A* | 85<br>62 | 12 (14%)<br>19 (30%) | 1.00<br>2.83 (1.24-6.47) |
| **Stage III only** | *C/C*<br>*C/A or A/A* | 72<br>53 | 11 (15%)<br>17 (32%) | 1.00<br>2.77 (1.15 – 6.66) |

Zhou et al, CCR 2005

# “Future Directions”

- Validation studies
  - Same disease site, same drug
  - Different disease site, same drug
- Other DNA repair gene polymorphisms
  - “Comprehensive” evaluations

# Since then…lung cancer validation?

| | | | |
|---|---|---|---|
| **XRCC1** | **7**<br>***3 Asian/4 Cauc.***<br>***(n= 36-248)*** | ***XRCC1Arg399Gln*** | **Platinum. *Gln/-* associated with worse GI toxicity in single Asian study[AOR 2.53 (1.06-6.03); *p*=0.03]. *Gln/Gln* worse survival in stage IIIA/B in US study, better survival in Italian study.** |
| | **3**<br>***2 Asian/1 USA***<br>***(n=36-229)*** | ***XRCC1 Arg194Trp;***<br>***XRCC1 Arg280His(single Asian study)*** | ***Arg/Arg* worse toxicity with gem/docetaxel in single study (*p*=0.03) .No tox. assoc. with cisplatin in 2nd study. No OS assoc.** |
| **XPD** | **7**<br>***2 Asian***<br>***(n=36-248)*** | ***XPD Asp312Asn;***<br>***XPD Lys751Gln;*** | **No assoc. in 5 studies.Variant genotype (*-312Asn/Asn*) worse OS in single study (p=0.003). *-751Lys/Lys* assoc. with Gr 4 neutropenia in one (*p*=0.02)** |

# ERCC1

| | | | |
|---|---|---|---|
| **ERCC1** | **7**<br>***3 Asian; 4 Cauc. (n=65-423)*** | ***ERCC1 118C/T*** | **Platinum. *C/C* better OS/RR in 3 Asian studies.**<br>**No associations in 4 studies in Caucasians.** |
| | **3**<br>***1 Asian; 2 USA***<br>***(n= 128-423)*** | ***ERCC1 8092C/A;*** | **Platinum. *C/C* had better OS and *A/-* had increased GI toxicity in US study** |
| | **1**<br>***China, n=162*** | ***ERCC1(262G/T;433T/C; 3525C/T; 4855C/T; 14443C/A)*** | **Small cell only + Carboplatin /VP16. *262T/T* worse OS [AHR 1.98;*p*=0.017].** |

# Reasons for lack of validation

- Heterogeneous populations
- No clear functional genomics data
- Small sample sizes
- “Fuzzy” hypothesis
- Multiple hypotheses
- “not a true matching validation set”

# Inherited Genetic Variation and Lung Cancer Outcomes

Horgan AM, Yang B, John T, Cescon D, Wheatley-Price P, Shepherd FA, Liu G.

- 237 genetic variations in 79 studies.
- Survival was the outcome in 89% of the studies
- Toxicity was outcome in 22%.
- Candidate polymorphisms in the DNA repair/synthesis pathway were the most frequently studied.
- Results were conflicting
- Many had little functional genomic data
- strong evidence supporting validation in large-scale confirmatory studies of any single polymorphism was lacking.
- Heterogeneity in study populations and inconsistencies in methodology between studies were common.
- Almost all were candidate polymorphism-based

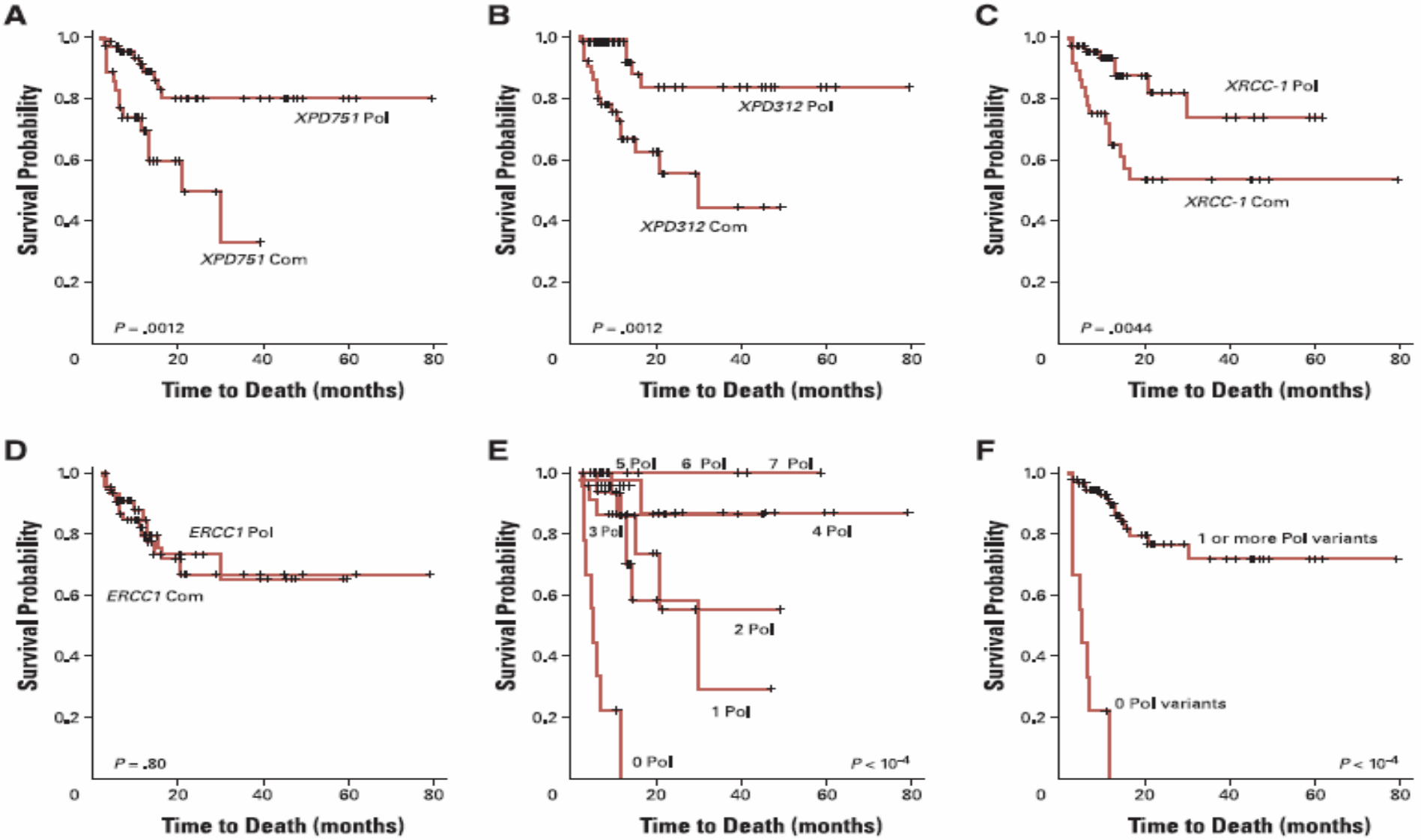
# Treatment Modalities

| | |
|---|---|
| **Radiation to Primary Tumor** | 66 (64%) |
| **1st line Chemotherapy Regimens** | |
| Carboplatin-Taxane | 63 (61%) |
| Cisplatin-Vinca Alkaloid | 21 (20%) |
| Cisplatin-Etoposide | 3 (3%) |
| Other Platinum Combinations | 8 (8%) |
| Non-Platinum 1st Line Agent | 8 (8%) |

# DNA-Repair Gene Polymorphisms Predict Favorable Clinical Outcome Among Patients With Advanced Squamous Cell Carcinoma of the Head and Neck Treated With Cisplatin-Based Induction Chemotherapy

*Miguel Quintela-Fandino, Ricardo Hitt, Pedro P. Medina, Soledad Gamarra, Luis Manso, Hernan Cortes-Funes, and Montserrat Sanchez-Cespedes*

*J Clin Oncol 24:4333-4339.*



# Reasons for discrepancy?

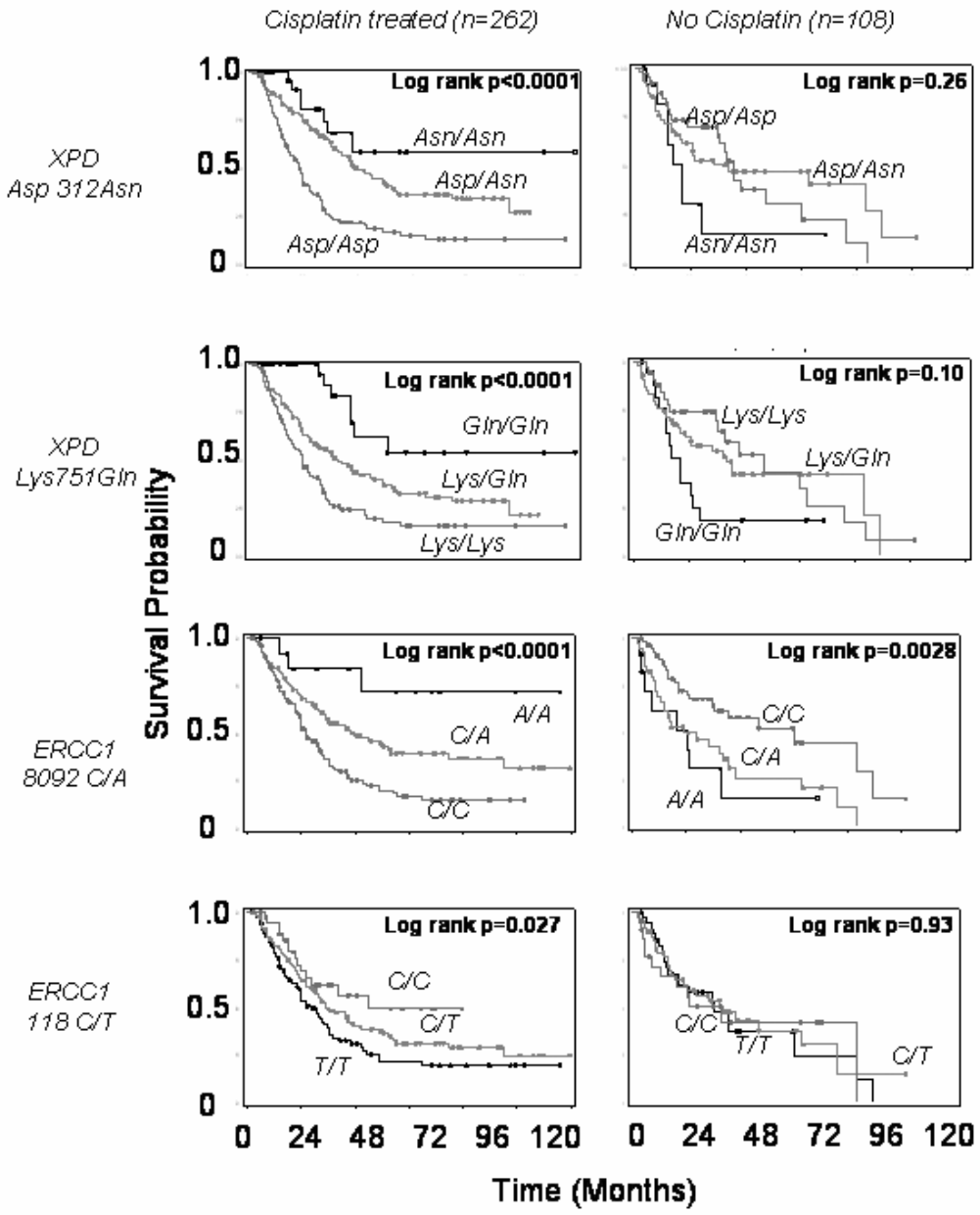
- Cisplatin vs carboplatin?
- Disease site specificity?

- False positive result(s)?
  - Small sizes
  - Heterogeneous populations (treatments)?

| Induction regimen* | N | % |
|---|---|---|
| CDDP + radiotherapy | 26 | 25.2 |
| CDDP + fluoropyrimidine | 31 | 30.1 |
| CDDP + fluoropyrimidine + taxane | 42 | 40.8 |
| Cisplatin + cetuximab | 4 | 3.9 |

DNA repair polymorphisms and Cisplatin-based and non-cisplatin treated esophageal Boston-based cancer patients

(Bradbury et al in preparation)



# Possible Validation Datasets

- Lung Cancer: BR.10, BR.24, TORCH, BRC4
- Head and Neck: HN.6
- Esophageal: RTOG, TROG

- Local observational datasets:
  - Lung Cancer (>300 with cisplatin treatment)
  - Head and Neck (>100 with cisplatin treatment)
  - Esophageal cancer (>100 with cisplatin treatment)

## Inherited Genetic Variation and Lung Cancer Outcomes

Horgan AM, Yang B, John T, Cescon D, Wheatley-Price P, Shepherd FA, Liu G.

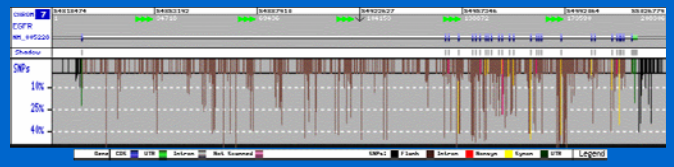
- Best candidates (at least 2 positive studies, any number of negative underpowered studies allowed)
  - *ERCC1 118C/T* in Asians
  - *EGFR intron 1* and *–216G/T* in EGFR treated patients
  - *GSTM1-null*
  - *p53Arg72Pro*
  - *MDM2309*

# Pharmacogenetic Example:

## EGFR polymorphisms and EGFR TKIs (2004-)

**Review of existing PK/PD/PG data**

**SNP - HapMap**



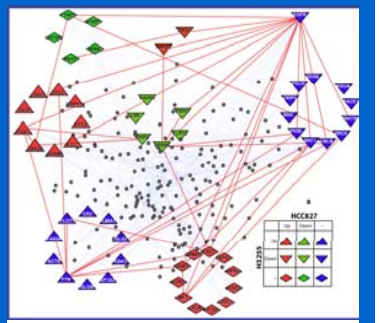
**Proprietary PK data**

**PGRN and public source PK/PG/PD data**

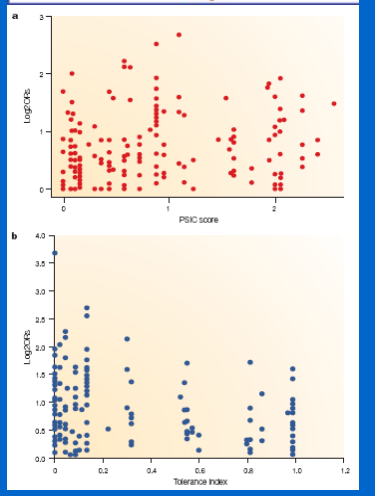
***In silico* and bioinformatic determination of best targets**

**Haploview/Tagger**

**I2D/PPI Networks**



**SIFT/PolyPhen/Coddle**

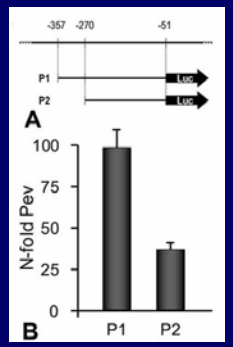


# Pharmacogenetic Example:
## EGFR polymorphisms and EGFR TKIs (2004-)

**Functional Assays** → **Identification of key targets to test in patient samples**

### Promoter Analysis
### AMPL

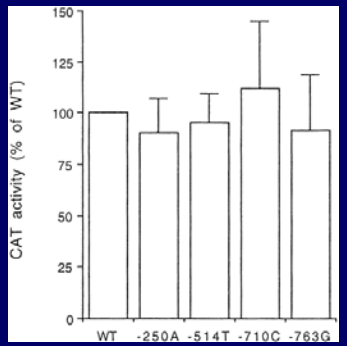
**Luciferase Promoter Assays**




**Liu et al, CR 2005**

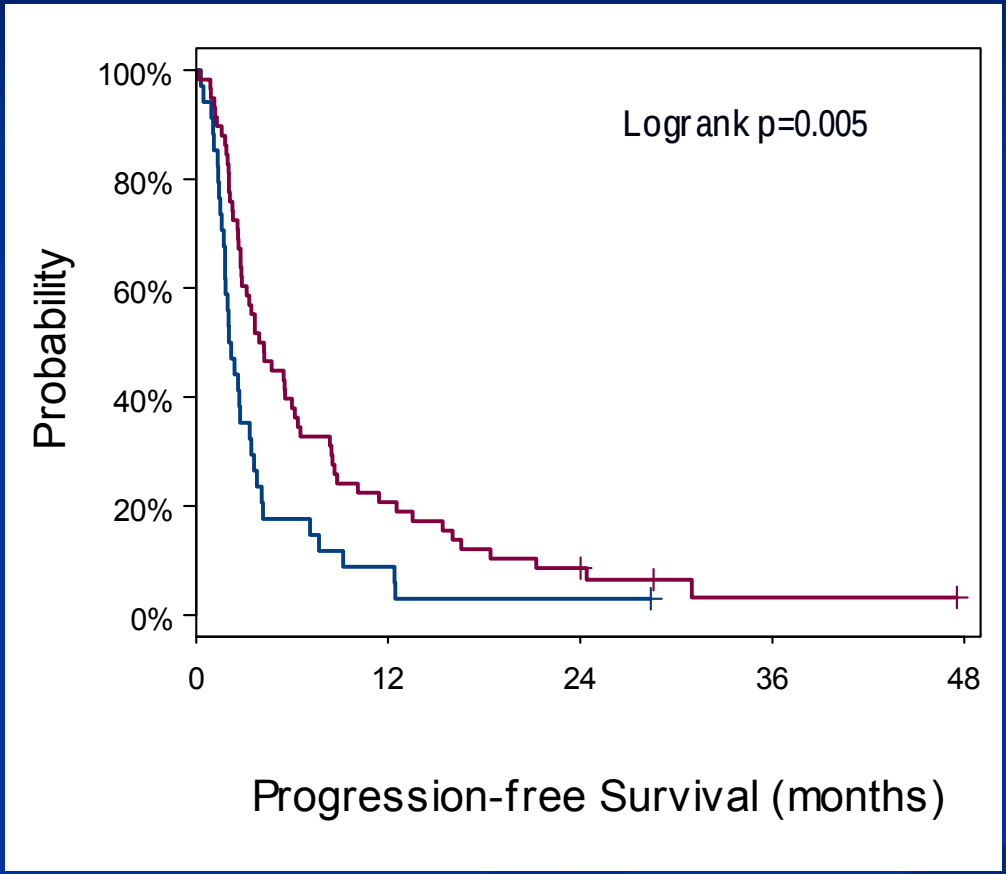
### Gene Expression/Binding Assays
### Collaboration with A. Adjei (Mayo/RPCI)/STTARR

**Haplotype Constructs and functional Binding and Expression assays**



# *-216G/T* polymorphism & PFS/OS

| | *T/-* | *G/G* |
|---|---|---|
| N (%) | 58(63%) | 34(37%) |
| Med PFS | 4.1 mos | 2.1 mos |
| Adj. HR | 0.62 | reference |
| 95%CI | (0.38-0.99) | |




Liu et al, TPJ 2007

| | | | |
|---|---|---|---|
| EGFR | **7**<br>*3 Asian, 4 Cauc*<br>*n = 70-173* | *EGFR intron 1 $(CA)_n$ Shorter/Longer* | **With Gefitinib**.No assoc. with OS in 5 studies. *Longer* assoc. with worse OS in single Asian study(*p*=0.039)<br>**No Gefitinib**: *Longer* associated with better OS in single US study (*p*=0.03) |
| | **1**<br>*Italy; n=124* | *ABCG2 421C/A* | **Gefitinib.***A/-* associated with diarrhea (*p*=0.0046) |
| | **4**<br>*1 Asian; 3Cauc*<br>*n= 92-170* | *EGFR -216G/T*<br>*EGFR -191C/A* | **Gefitinib:** *T* allele of *-216* better PFS alone or in combination with Intron 1*S/S* in US study.Combination assoc. with better OS (*p*=0.02). *EGFR1 GC* haplotype worse OS (*p*=0.015) but only when analysis restricted to stages 0 and 1in 2nd study. |

# Possible Validation Studies

EGFR TKI treated patients

- Lung Cancer
  - BR.21
  - BR.19
  - TORCH
  - BRC4
- Head and Neck Cancer
  - HN.6

Lung Cancer General prognosis (*GSTM1, p53, MDM209*)

- BR10+BR19 no treatment arms, BR24 both arms

*Minireview*

Systematic Validation Approach

# Genetic Polymorphisms and Head and Neck Cancer Outcomes: A Review

Cancer Epidemiol Biomarkers Prev 2008;17(3). March 2008

Jessica Hopkins,[1,2] David W. Cescon,[2,3] Darren Tse,[2] Penelope Bradbury,[2] Wei Xu,[4] Clement Ma,[4] Paul Wheatley-Price,[2] John Waldron,[5] David Goldstein,[6] Francois Meyer,[7] Isabelle Bairati,[7] and Geoffrey Liu[2,8]

- 24 polymorphisms had at least one positive association with outcomes in HNC
- All 24 and one since published are being validated in 540 early stage HNC patients all treated uniformly with radiation.
  - Using data/specimens from Phase III Secondary prevention study of AT/BC.

# To the extreme → Exploratory candidate polymorphism array chip

- 520 esophageal cancers from Boston
  - All stages
  - All treatments
- 1536 Candidate polymorphisms in various cancer-related, oncogene, tumor suppressor, cell cycle, apoptotic, xenobiotic metabolism and pharmacogenetic pathways chosen from polymorphism literature of upper aerodigestive cancers
- Validation in Toronto samples +/- RTOG? +/- TROG?

# 2. Tagging Approach

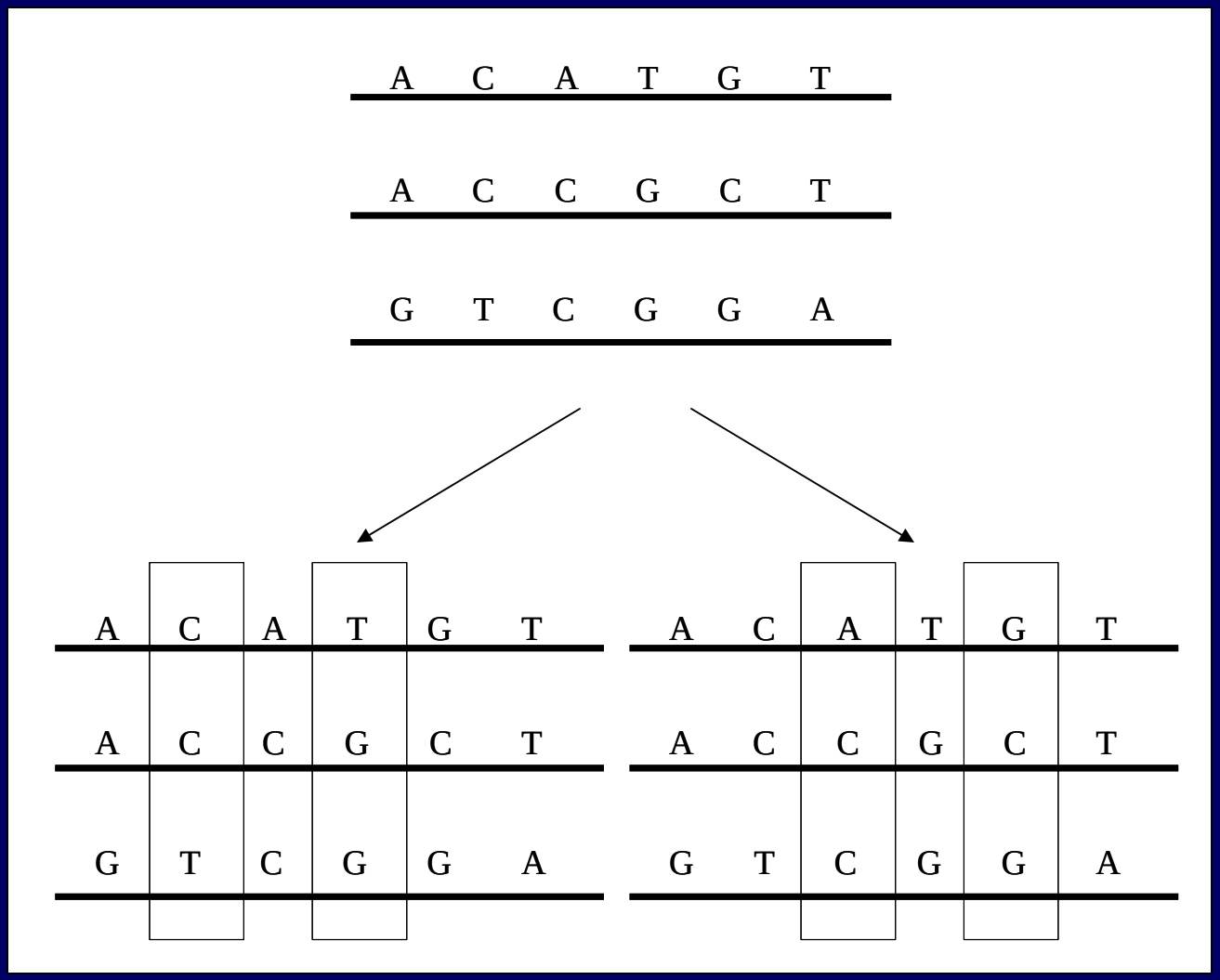
- Tag/Block analysis
  - One SNP ≠ function
  - Utilizes LD structure to reduce number of polymorphisms required to be genotyped to identify most/all of the common genetic variation in a gene

  - Still need to pick the genes of interest
  - Potential misclassification since most haplotypes are inferred from computer programs

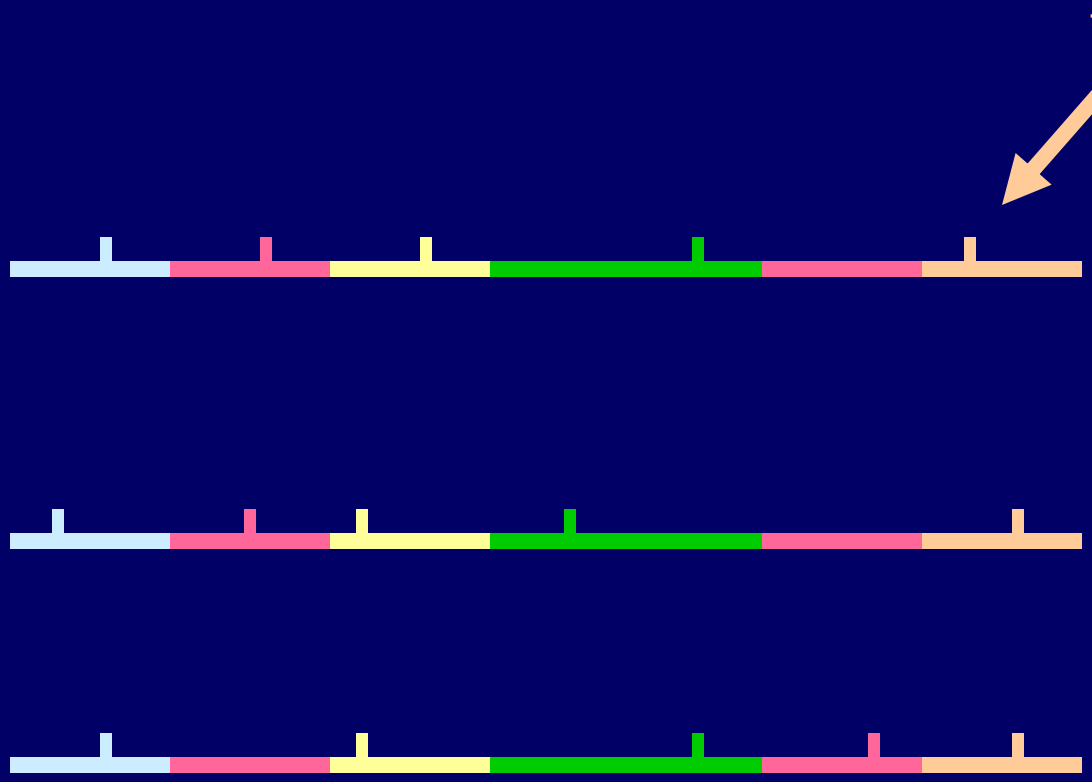
# Haplotypes and Tagging

- Multiple SNPs located close together .
- Haplotype blocks are smallest segments of DNA containing SNPs that tend to be conserved without recombination and inherited as a unit
- Haplotype analysis = analyze the block rather than a single SNP

# Reducing the number of markers In Haplotypes

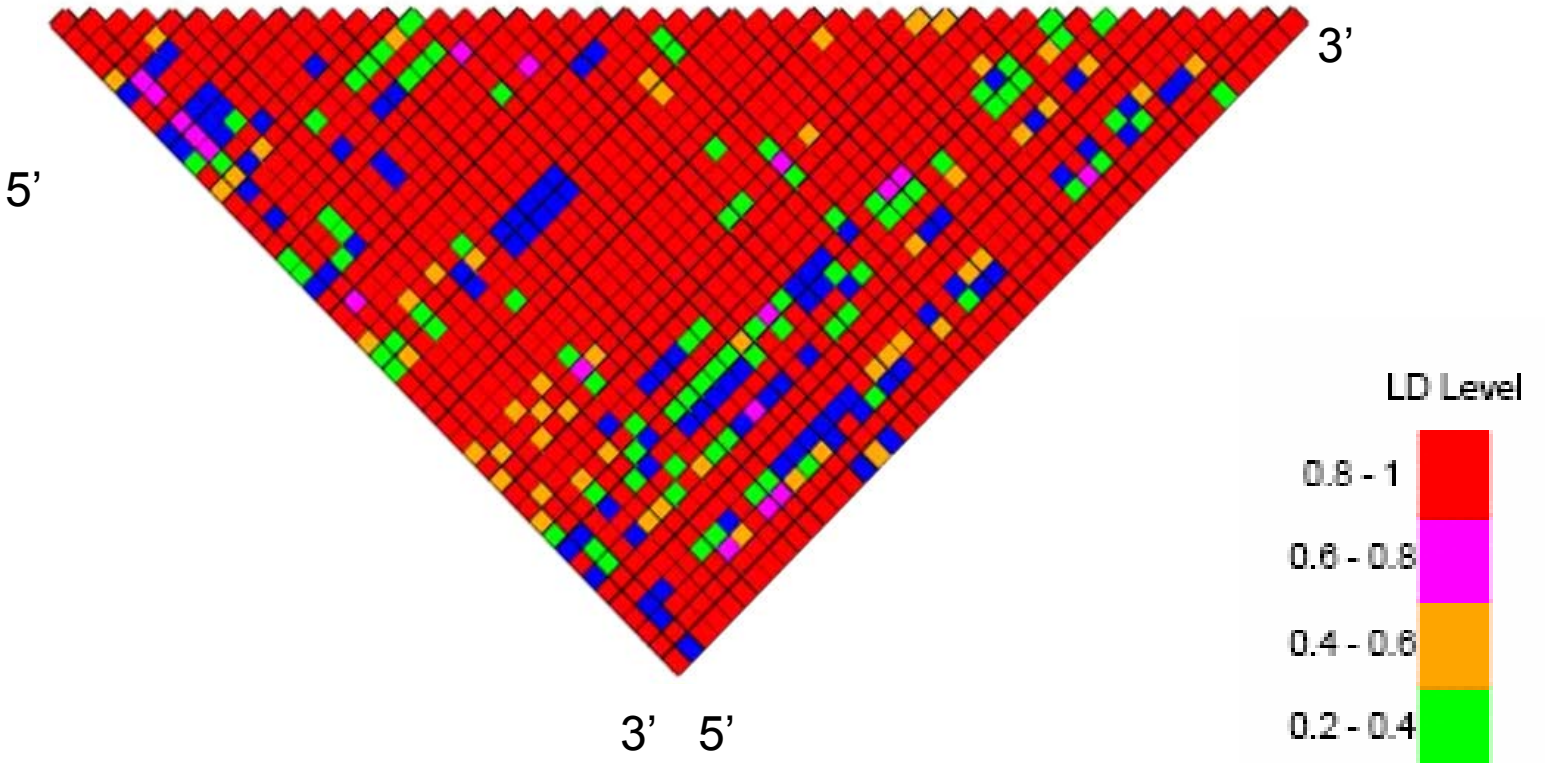


# Using 5 TagSNPs to define variation in a gene




Each colour is a different haplotype block

m




A graphical depiction of Caucasian-American EGFR linkage disequilibrium within the area resequenced. Pairwise D' values’are shown (*P*< 0.05).

# Tagging

- Hard to do using archival FFPE samples (too much DNA)
- Exploratory

- Easier to do using blood
  - TORCH
  - BRC4/MARVEL
  - BR.24

# TagSNPs

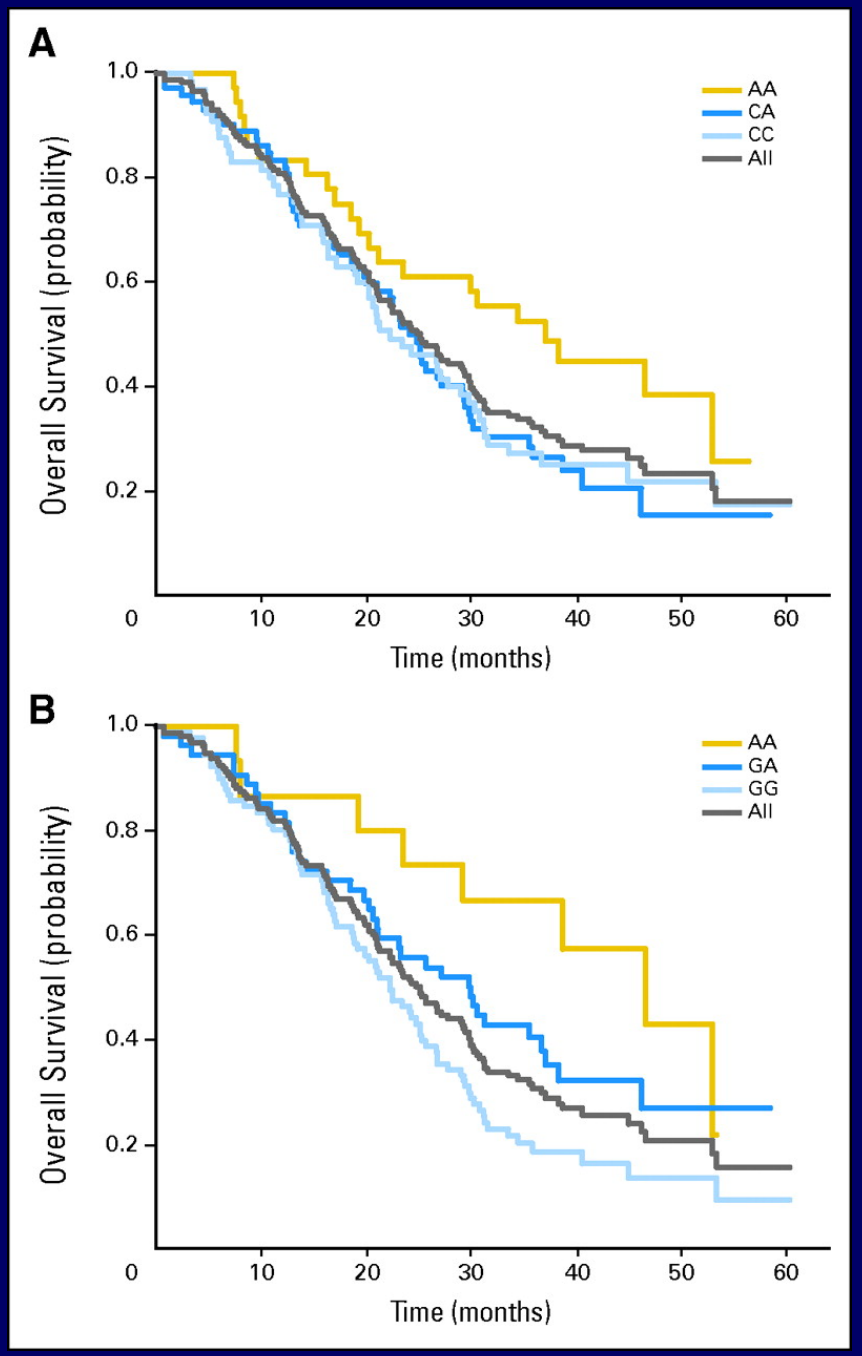
- TagSNP has association with outcome
- In silico functional evaluation of SNPs in LD with TagSNP
- Deep resequencing to identify new polymorphisms in LD → In silico functional evaluation
- Biological functional evaluation → often partnered with site known to have the constructs, etc. If not, will need to develop

- Replicate/validate in other datasets

# Combined TagSNP and candidate SNP selection approaches

# Association of Vascular Endothelial Growth Factor and Vascular Endothelial Growth Factor Receptor-2 Genetic Polymorphisms With Outcome in a Trial of Paclitaxel Compared With Paclitaxel Plus Bevacizumab in Advanced Breast Cancer: ECOG 2100

Bryan P. Schneider, Molin Wang, Milan Radovich, George W. Sledge, Sunil Badve, Ann Thor, David A. Flockhart, Bradley Hancock, Nancy Davidson, Julie Gralow, Maura Dickler, Edith A. Perez, Melody Cobleigh, Tamara Shenkier, Susan Edgerton, Kathy D. Miller JCO Oct 2008

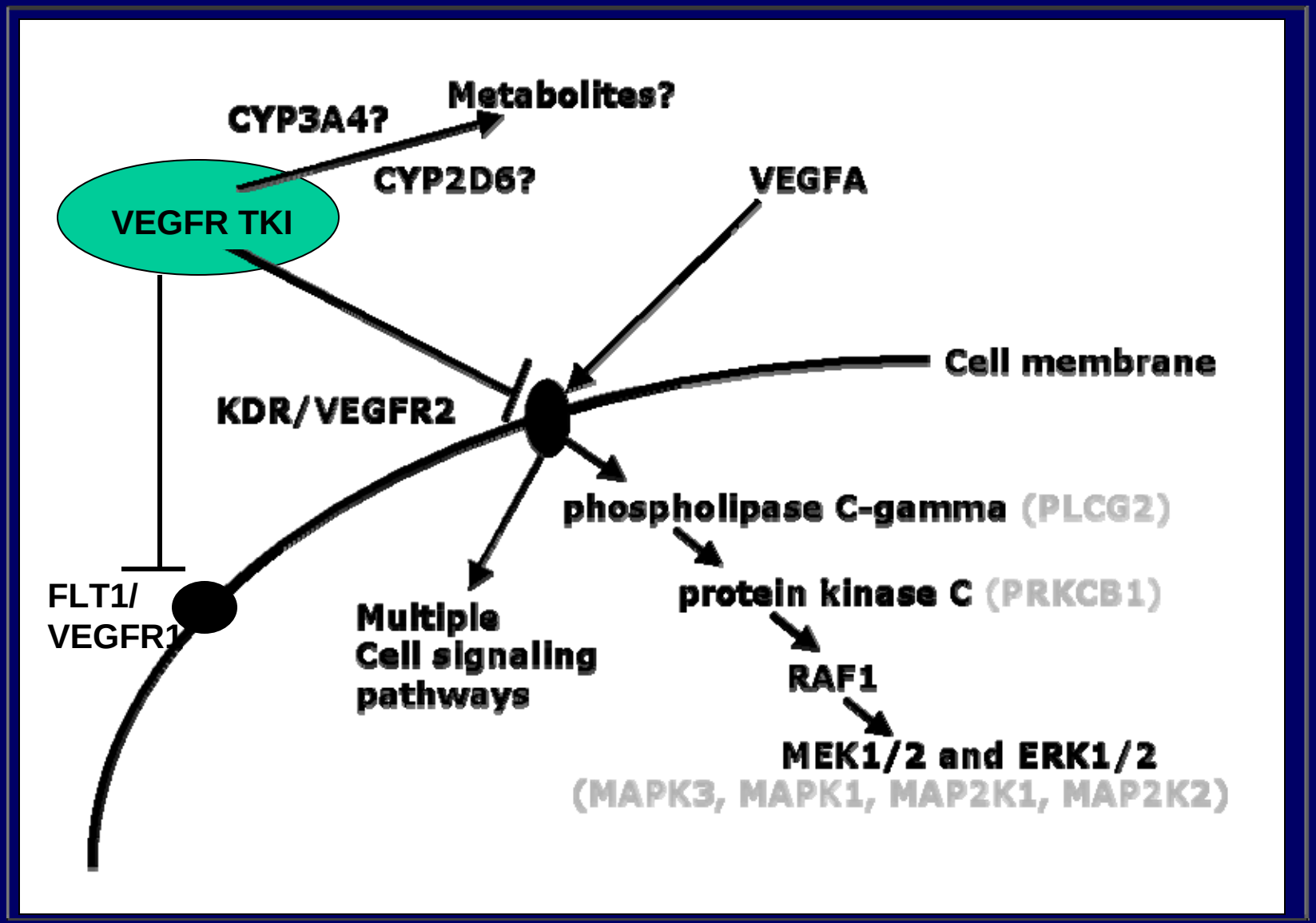



*(A) VEGF-2578 C/A*
*(B) VEGF-1154 G/A.*

**Table 4.** Relationship of *VEGF* Genotype With Grade 3 or 4 Hypertension

| Single Nucleotide Polymorphism | Patients | | % of Patients With Grade 3 or 4 Hypertension | *P* |
|---|---|---|---|---|
| | No. | % | | |
| *VEGF-634* | | | | |
| CC | 27 | 15.3 | 0 | .013 |
| GC | 82 | 46.3 | 22 | |
| GG | 68 | 38.4 | 19 | |
| CC *v* GC + GG | | | | .005 |
| *VEGF-1498* | | | | |
| TT | 60 | 33.9 | 8 | .056 |
| CT | 82 | 46.3 | 22 | |
| CC | 35 | 19.8 | 23 | |
| TT *v* CC + CT | | | | .022 |
| *VEGF-2578* | | | | |
| AA | 36 | 20.8 | 22 | .32 |
| CA | 72 | 41.6 | 21 | |
| CC | 65 | 37.6 | 12 | |
| CC *v* CA + AA | | | | .16 |
| *VEGF-1154* | | | | |
| AA | 15 | 9.4 | 27 | .29 |
| GA | 54 | 38.8 | 22 | |
| GG | 91 | 56.9 | 14 | |
| GG *v* GA + AA | | | | .15 |

Abbreviation: *VEGF*, vascular endothelial growth factor.




VEGFR TKI Pathway Approaches

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ISSN 0735-1097/07/$32.00
doi:10.1016/j.jacc.2007.04.074

# Polymorphisms of *KDR* Gene Are Associated With Coronary Heart Disease

Yibo Wang, PHD,* Yi Zheng, MD,* Weili Zhang, PHD,* Hui Yu, MS,* Kejia Lou,* Yu Zhang,* Qin Qin, MD,† Bingrang Zhao, MD,† Ying Yang, MD,‡ Rutai Hui, MD, PHD*

*Beijing, Tianjin, and Shandong, People's Republic of China*

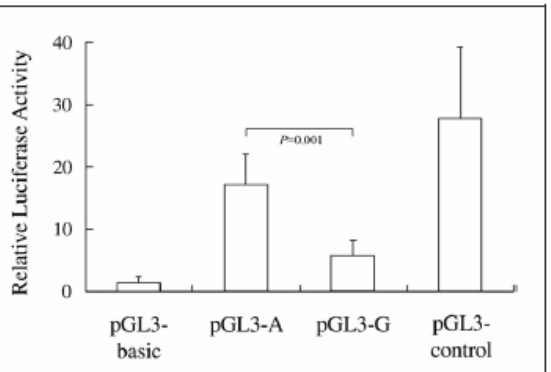



**Figure 1** SNP-604C–Bearing *KDR* Promoter Had Lower Transcription Activity

The pGL3 luciferase reporter contained either the T (pGL3-T) or C allele (pGL3-C) at the promoter −604 locus. Values represent the average of 6 experiments and the bars represent the standard deviation. The pGL3-basic was used as a negative control without any promoter sequence, and pGL3-control as a positive control. KDR = kinase insert domain-containing receptor; SNP = single nucleotide polymorphism.

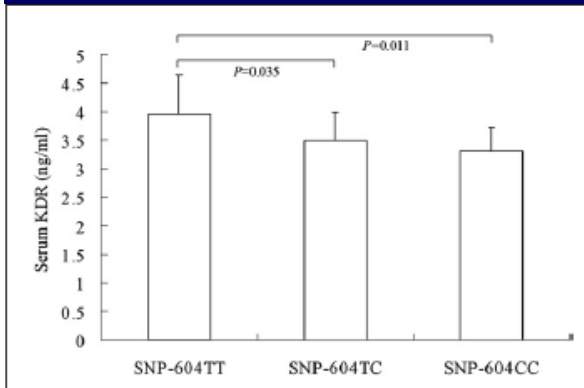



**Figure 3** Serum Levels of KDR Antigen Were Correlated With Genotypes of SNP-604

Levels of the serum KDR were presented as means, and the T bars represented standard deviations. The correlation was significant (p = 0.013), and Spearman coefficient for the existing correlation was rs = −0.374. Nineteen samples with TT genotype, 14 with TC genotype, and 10 with CC genotype were analyzed. Abbreviations as in Figure 1.

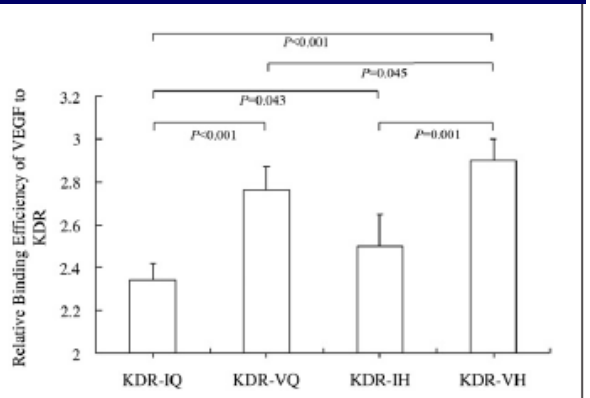


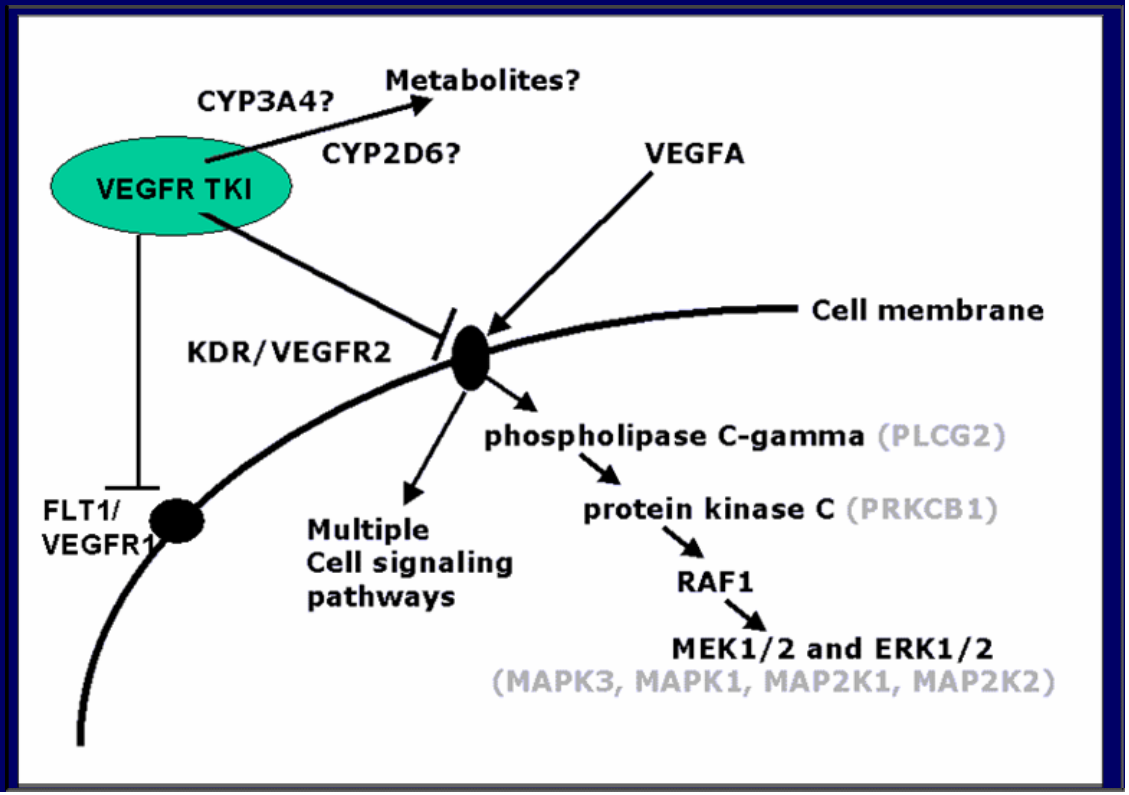

**Figure 2** SNP1192 and SNP1719 Influenced the Binding Efficiency of VEGF to KDR

HEK293s cells were transfected with 8 μg of pcDNA3.1-KDR (KDR-VH, KDR-IH, KDR-VQ, or KDR-IQ). After 36 h, the cells were rinsed with cold phosphate-buffered saline 3 times, and the binding of vascular endothelial growth factor $(VEGF)_{165}$ (10 ng/ml, R&D Systems, Minneapolis, Minnesota) was carried out in binding buffer containing DMEM, 25 mmol/l HEPES (pH 7.4), 1 μg/ml heparin, and 0.1% gelatin for 2 h at 4°C. Then the cells were rinsed with cold phosphate-buffered saline 5 times, lysed with cell lysis buffer, followed by enzyme-linked immunosorbent assay. The values were the ratio of VEGF to KDR. The experiments were repeated 3 times, and 2 replicates were performed for each experiment. The values were presented as means, and the T bars represented standard deviations. Abbreviations as in Figure 1.

| Gene | Drug | Literature polymorphism | Number of tagSNPs |
|---|---|---|---|
| | | Tier 1 | Tier 2 |
| ***VEGF(A)*** | **VEGF TKI** | *–2578C>A; -1498C>T; -1154G>A; -634G>C; -460C>T; +405G>C; +936C>T* | 20 |
| ***KDR/VEGFR2*** | **VEGF TKI** | *-604C>T; +4422 AC repeat; V297I; Q472H* | 24 |
| ***FLT1/VEGFR1*** | **VEGF TKI** | *C519T (GenBank D64016)* | 41 |

# Pathway analyses

- “Global pathway”
- Equally weighted pathways
- Weighted pathways



# 3. Genome-wide approach

- Microarray “Chip” technology
- Non-hypothesis driven
- Hypothesis generation
- Multiple comparisons – potential false positive associations
  - Costly
  - Needs multiple replications/validations in other datasets
  - Developmental bioinformatics and high dimensionality biostatistics required (techniques in development currently)

# Head and Neck Cancer Radiation Outcomes Study (co-PIs Liu/Meyer)

- 540 HN cancers from Quebec in completed Phase III study of secondary prevention using alpha-tocopherol/beta-carotene
- DNA extracted/mature clinical outcomes data

- Toronto observational dataset validation?
- HN.6 validation?

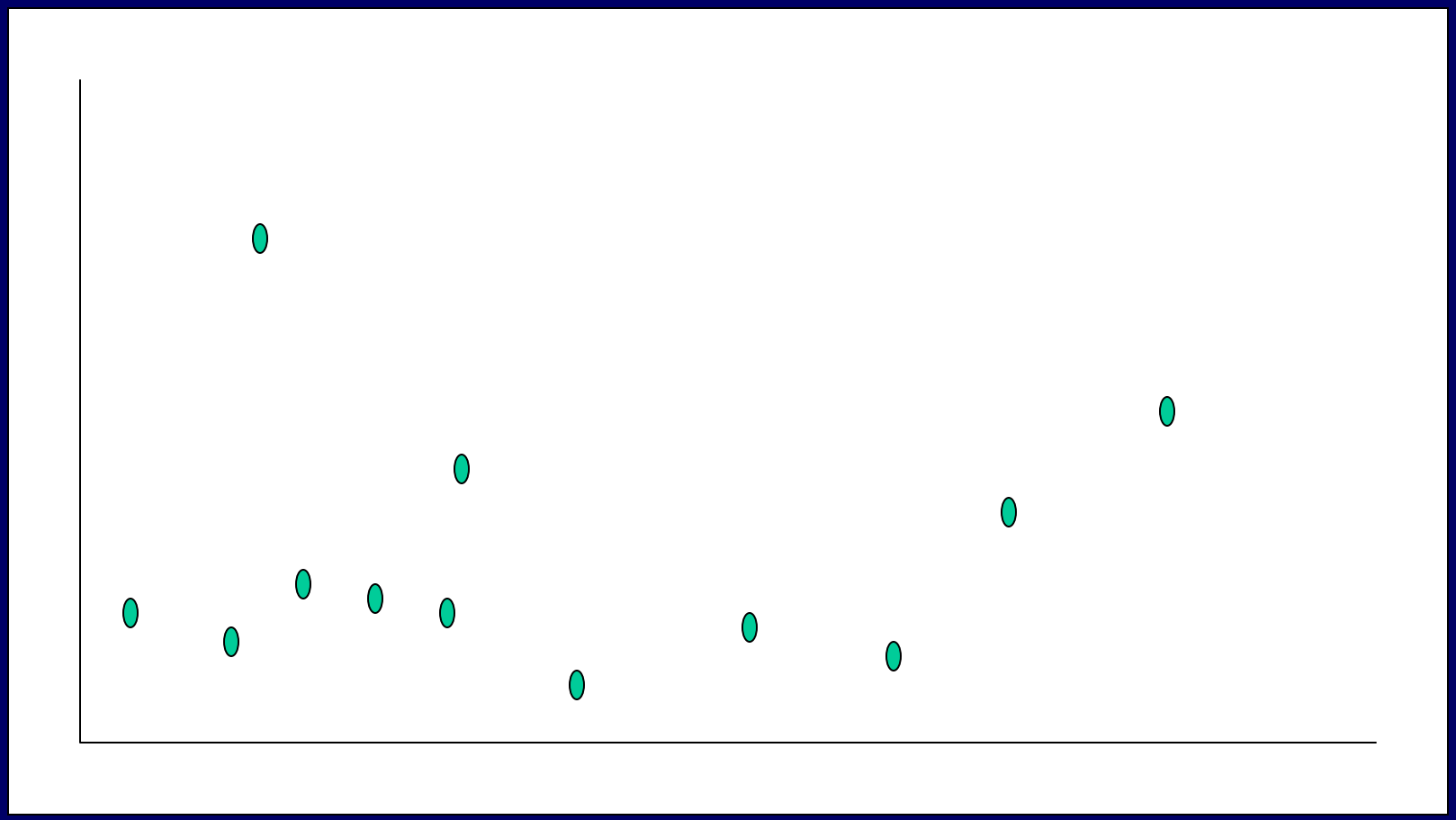
# Toronto Lung Cancer GWAS dataset

(PI – Hung/co-PI Liu)

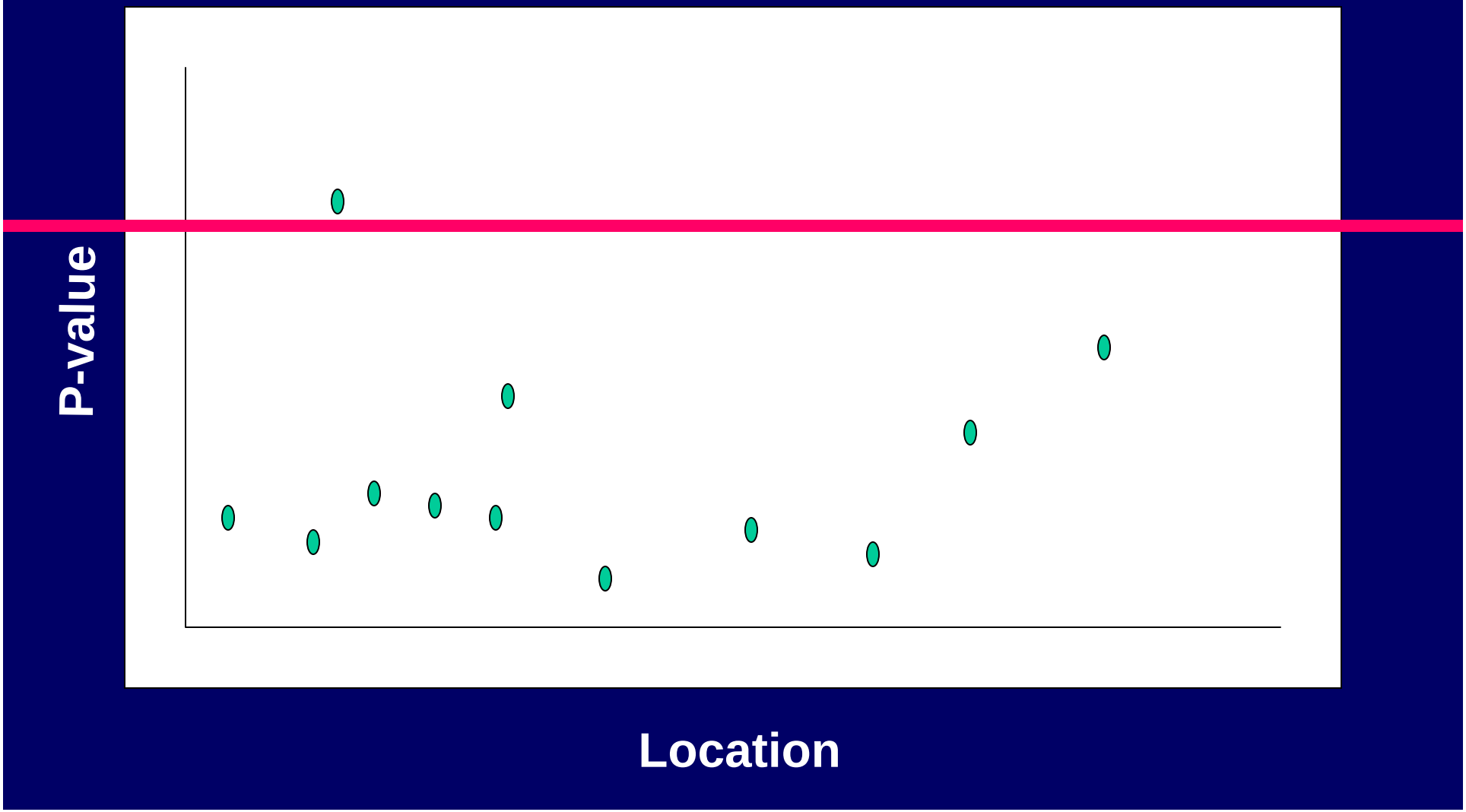
- 419 Caucasians with Lung Cancer GWAS
- All stages and treatments
- CCO survival data
- Anne Horgan working on outcomes

- BR.24 validation?
- Boston validation?
- PMH validation?

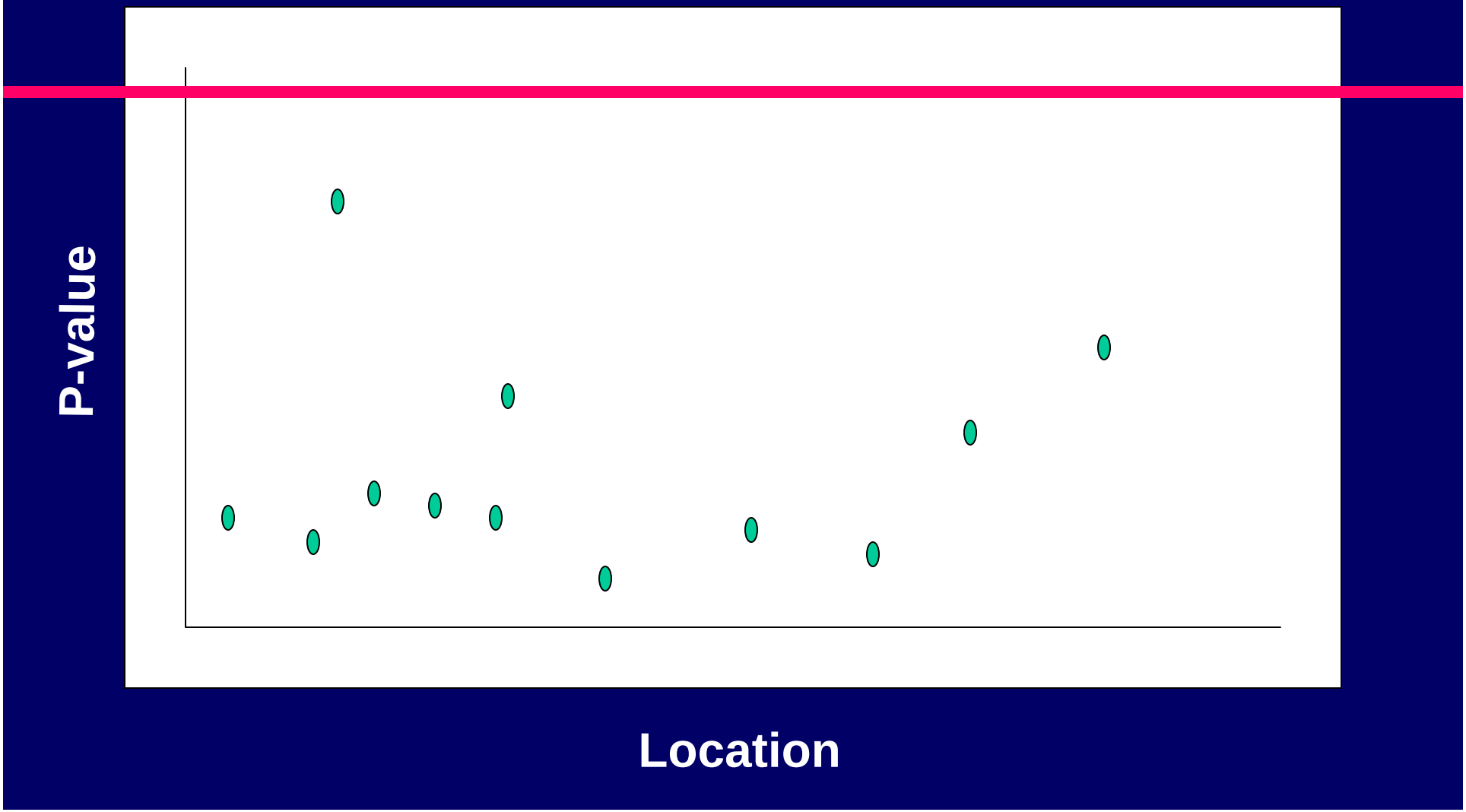
# Cutpoints



# Cutpoints



# Cutpoints



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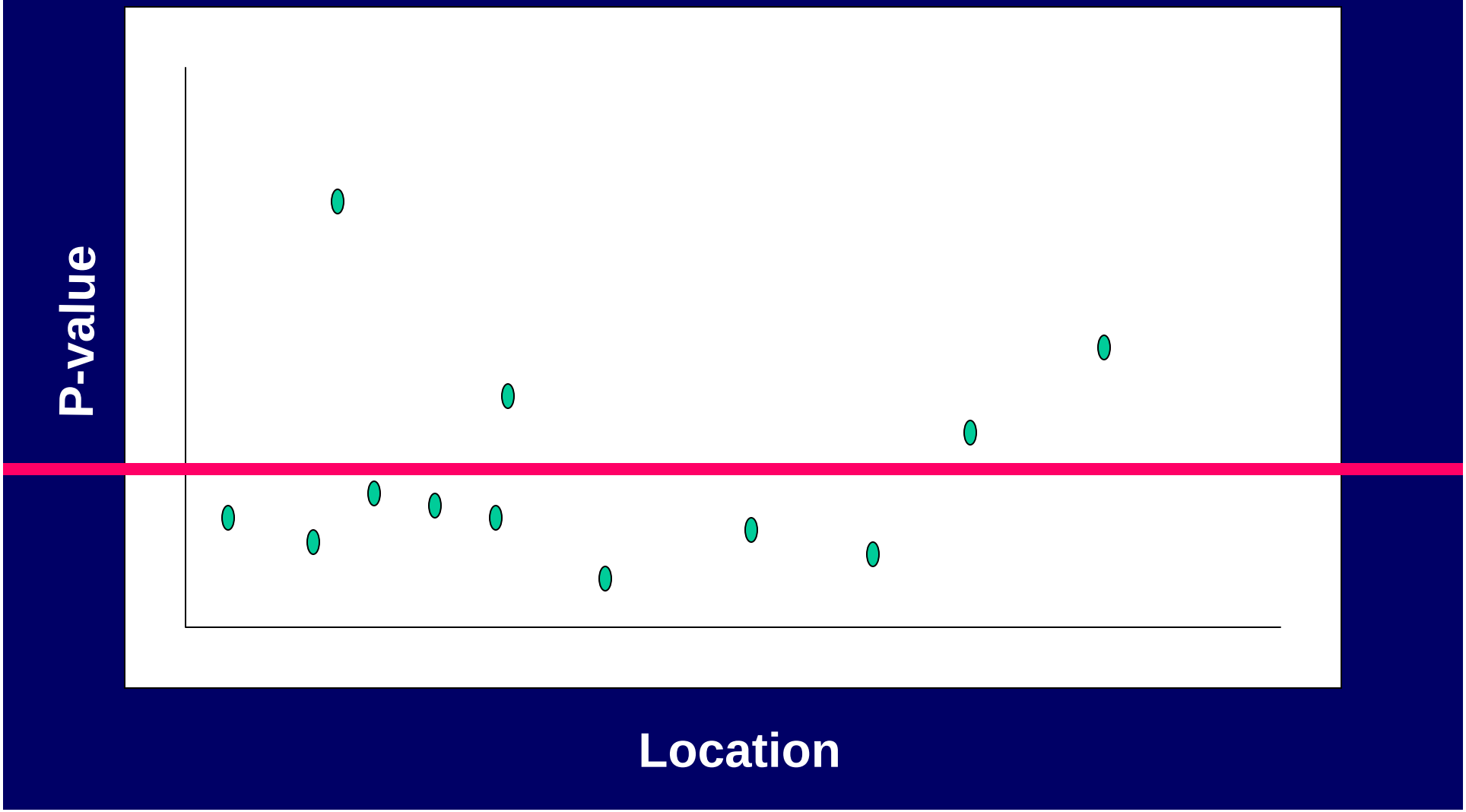
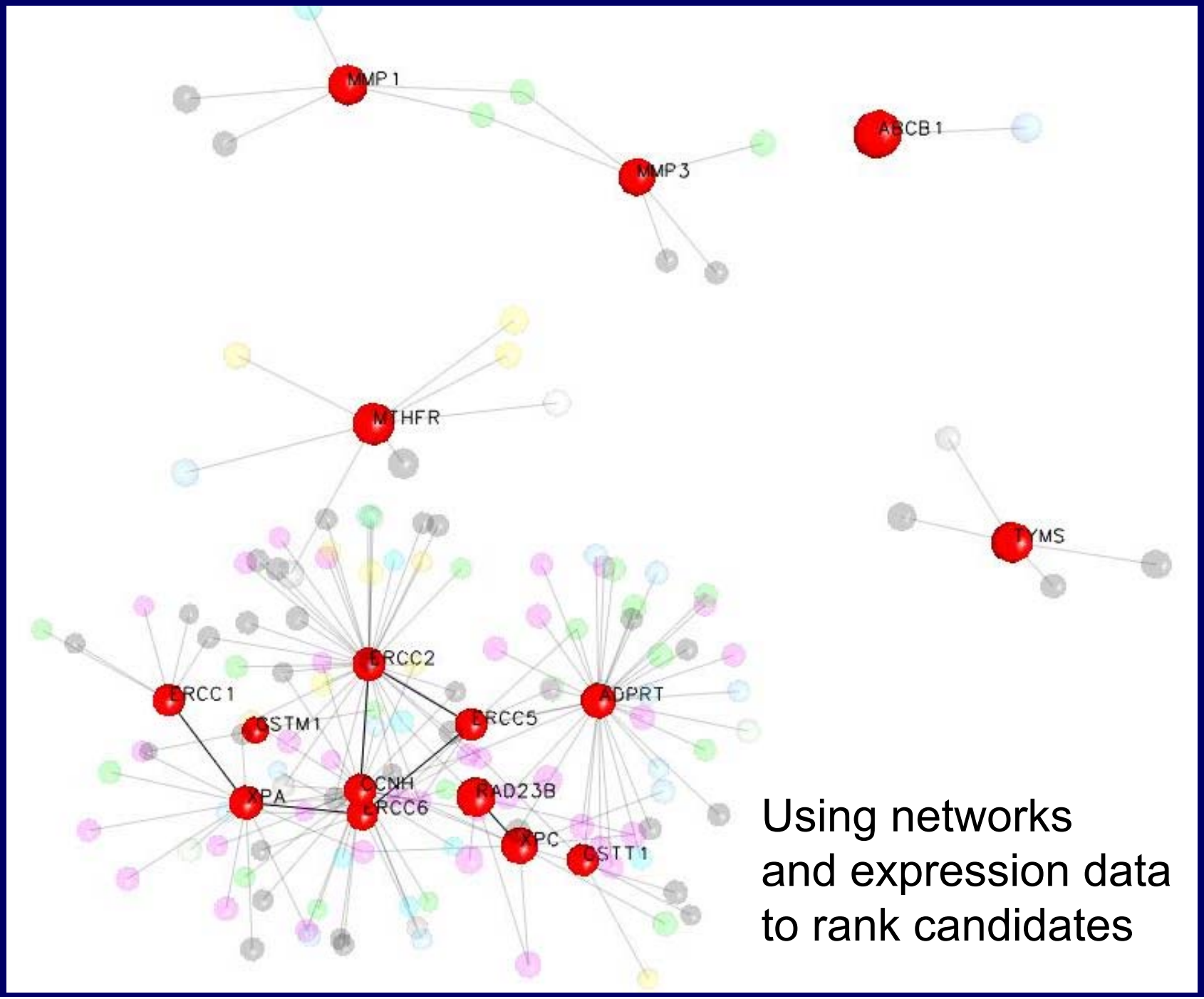
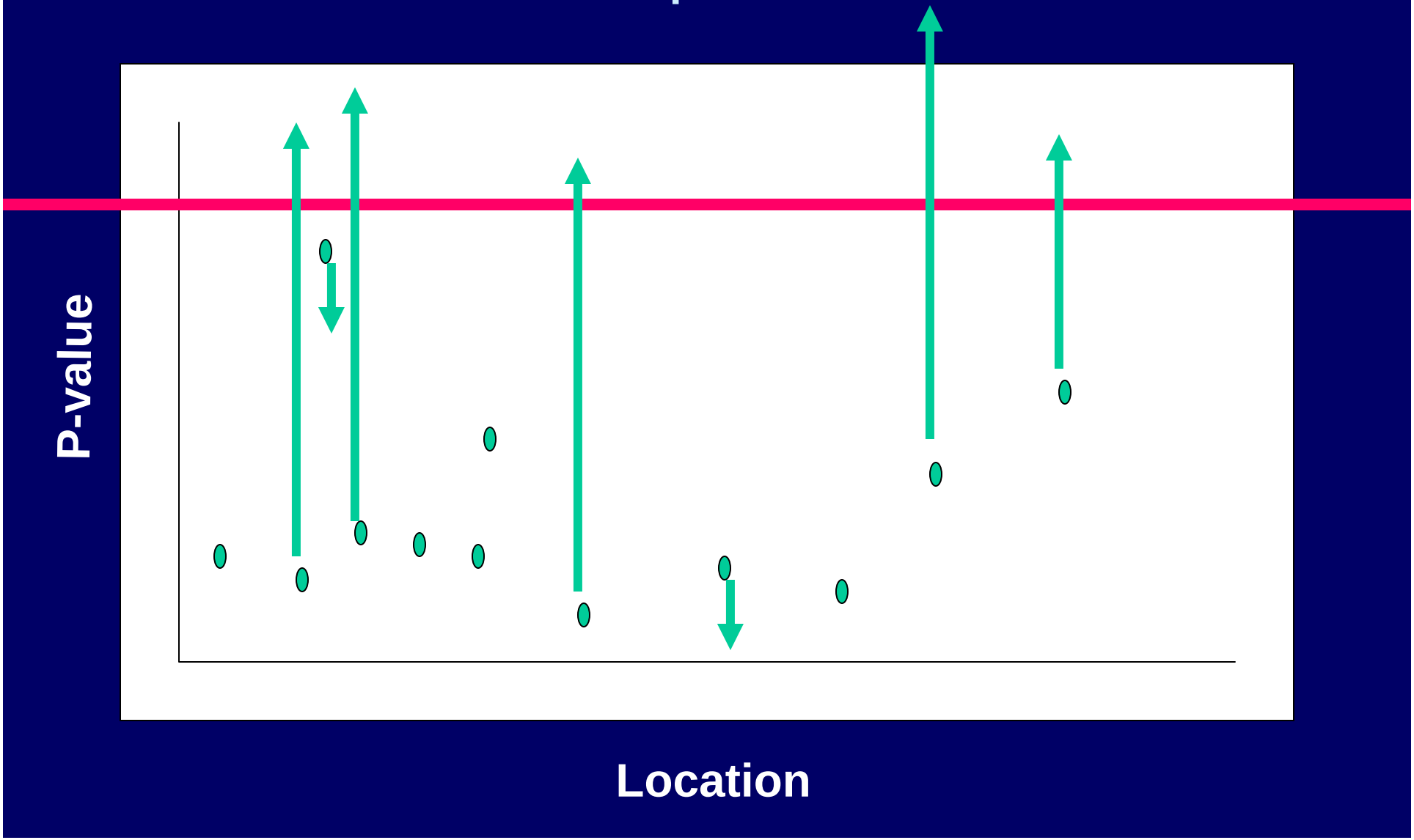





Using networks and expression data to rank candidates

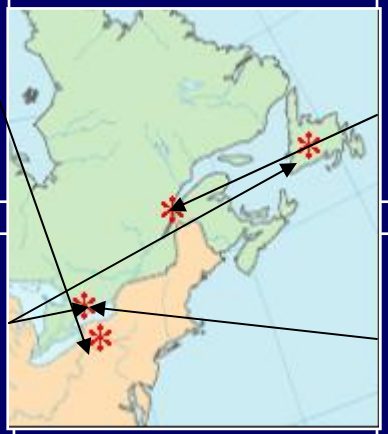
To Bioinformatically-inform the weighting of data

# Cutpoints



# Pharmacogenetic Epidemiology of Vitamin D in Head and Neck Cancer Outcomes

Vit D Resequencing data (A. Adjei, Roswell Park CI)



Samples, Serum, and Epidemiology (F. Meyer, I Bairati, P. Douville, Laval)

Bioinformatics analysis (I. Jurisica, PMH and S. Savas, Memorial)

Genotyping,Epidemiology and Analysis (G. Liu, W. Xu, PMH)

Multi-institutional, International Collaboration using Global, Unweighted and Weighted Pathway Analyses of Combined Candidate Polymorphism + Tagging Approaches + Secondary analysis of GWAS

# Summary

- Traditional Candidate polymorphism selection requires
  - Rigorous functional genomic evaluations
  - Multiple validation datasets
- Pathway and Tagging Approaches may be more helpful
- GWAS has both potential benefits but limitations
- ? Bioinformatically informed analysis

# Main Laboratory

# The “Teams”

**Liu Clinical Team**
- Penny Bradbury
- Paul Wheatley-Price
- Anne Horgan
- Tom John
- Dave Cescon
- Jessica Hopkins

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- Ming Tsao
- Rebecca Wong
- Gail Darling
- Drew Hope
- Brian O’Sullivan
- John Waldron
- Jonathan Irish
- Fei-Fei Liu
- Natasha Leighl
- Suzanne Kamel-Reid
- Andrea Bezjak
- David Hedley
- Suzanne Kamel-Reid
- Ron Feld
- Jennifer Knox
- Tom Waddell
- Shaf Keshavjee + many more

**Liu Lab Team**
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- Darren Tse
- Devalben Patel
- Kevin Chan
- Dangxiao Cheng
- Sevtap Savas
- Nicole Perera
- Marjan Emami
- Azad Kalam
- Joe Geraci (Bioinformatics)
- Jianbao Wu (Biostatistics)
- Crystal Johnston

**Bioinformatics/Biostatistics**
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- Harvard (Xihong Lin)

**Roswell Park Collaborators**
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- Mary E. Reid

**Genome Quebec Collaborators**
- Sharon Marsh

**NCIC Collaborators**
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- Karen Gelmon
- Lillian Siu/Amit Oza/Eric Chen
- Joe Pater
- Judy-Anne Chapman
- Keyue Ding
- Stephen Chia

**CHUQ/Laval Collaborators**
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- Francois Meyer
- Elodie Sampson

**Harvard Collaborators:**
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- Kofi Asomaning
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- Matthew Kulke
- Li Su
- Mike Wang
- Rebecca Heist

**Lunenfeld Collaborators**
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