Identification of Prognostic Genes, Combining Information Across Different Institutions and Oligonucleotide Arrays

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Introduction

- **CAMDA: "Critical Assessment of Microarray Data Analysis"**

- **CAMDA Challenge:** Pool information across studies to yield new biological insights.

- Our focus:
  1. **Adenocarcinoma** histology
  2. **Survival** outcome.
  3. **Michigan** (Beer, et al. 2002 *Nature Med*) & **Harvard** (Bhattacharjee, et al. 2001 *PNAS*)

Introduction

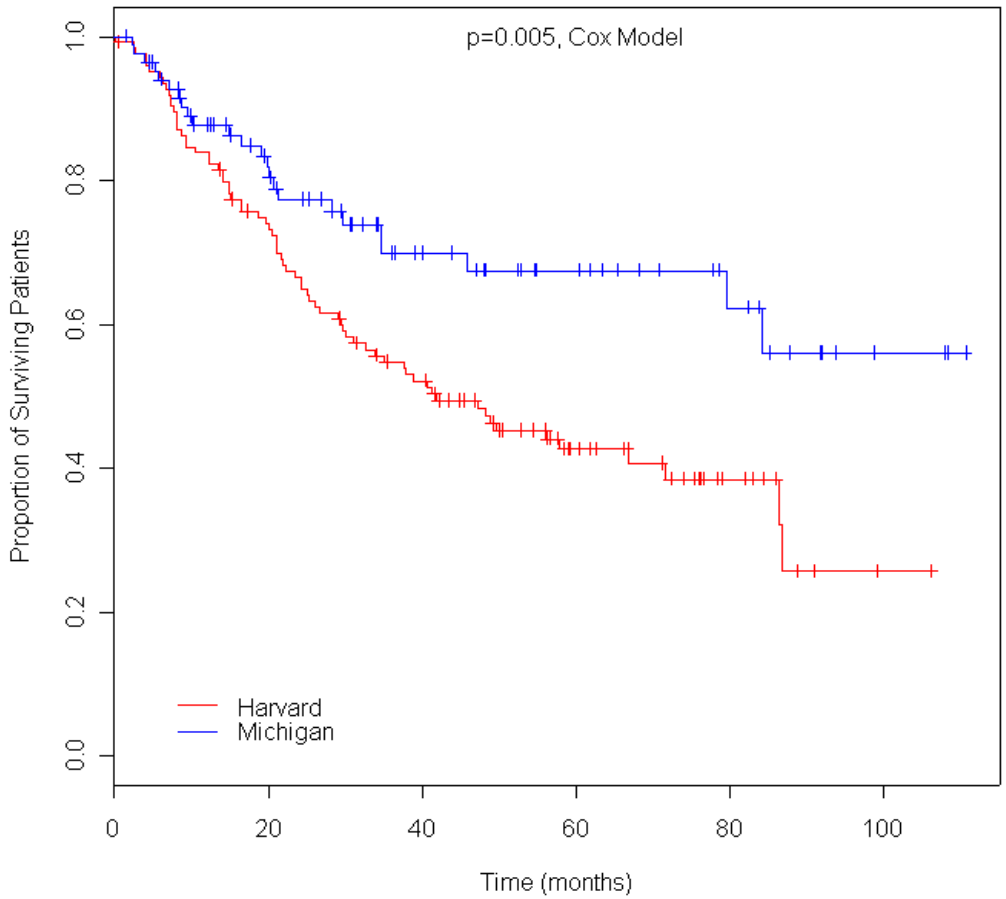
Our goals:

1. **Pool information** across different studies to **identify prognostic genes** for lung adenocarcinoma patients.

   - Offer information on patient survival ***over and above*** the information already provided by readily available clinical predictors.

2. Develop methodology to **pool information across different versions of Affymetrix chips** in such a way that we obtain comparable expression levels across the different chip types.

Pooling Information Across Studies



- Comparable distributions of age, gender, stage, smoking status, and follow-up time.
- Different survival distributions
- Fixed study effect included in our survival models to account for this heterogeneity

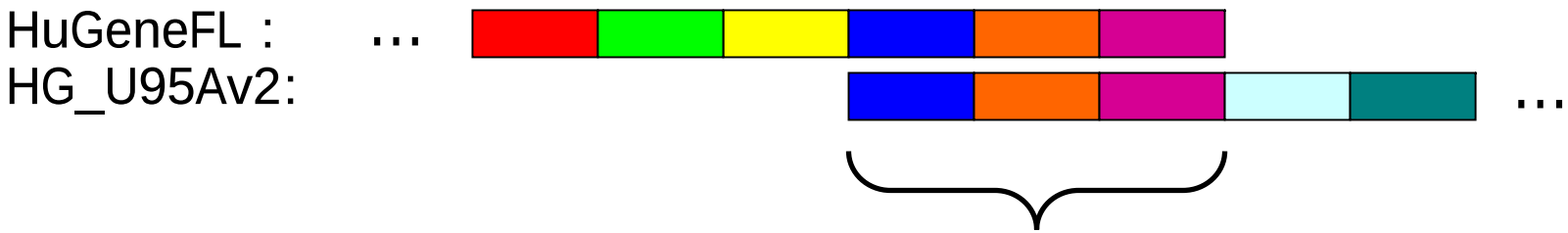
Pooling Information Across Chip Types

- Two studies used different chip types:
  - **Michigan**: **HuGeneFL**

    6,633 probesets/20 probe pairs each
  - **Harvard**: **U95Av2**

    12,453 probesets/16 probe pairs each
- Standard analyses on Affy-determined probesets not expected to yield comparable quantification

Pooling Information Across Chip Types



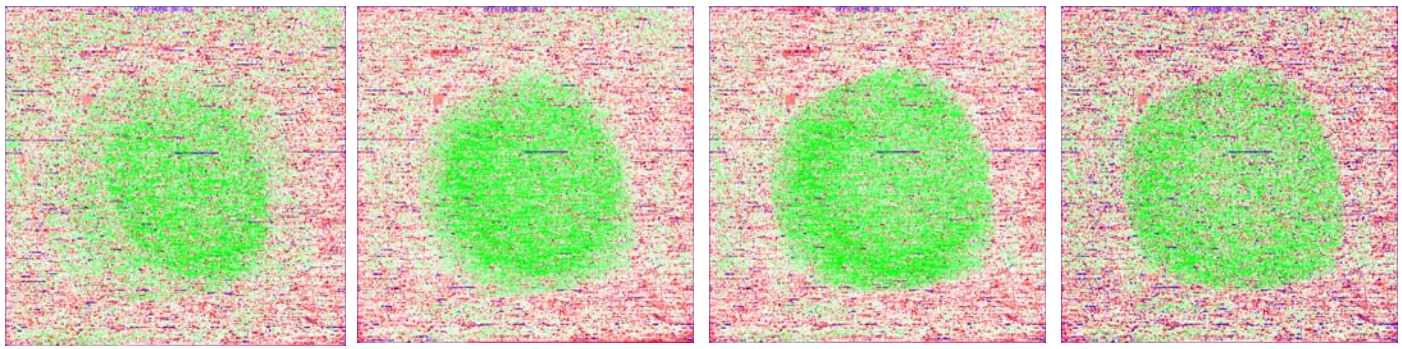
Our Solution

1. Identify "**matching probes**"
2. **Recombine** into new probesets based on UNIGENE clusters, which we refer to as "partial probesets"
3. **Eliminate** any probesets containing just one or two probes

- **Result: 4,101 partial probesets**.

Quality Control

- Several poor quality arrays removed
  - Large dead spot on center of 4 Michigan chips



L54 L88 L89 L90

  - 6 other Michigan chips/2 Harvard chips removed
- Matching clinical/microarray data for 200 patients (124 H, 76 M)

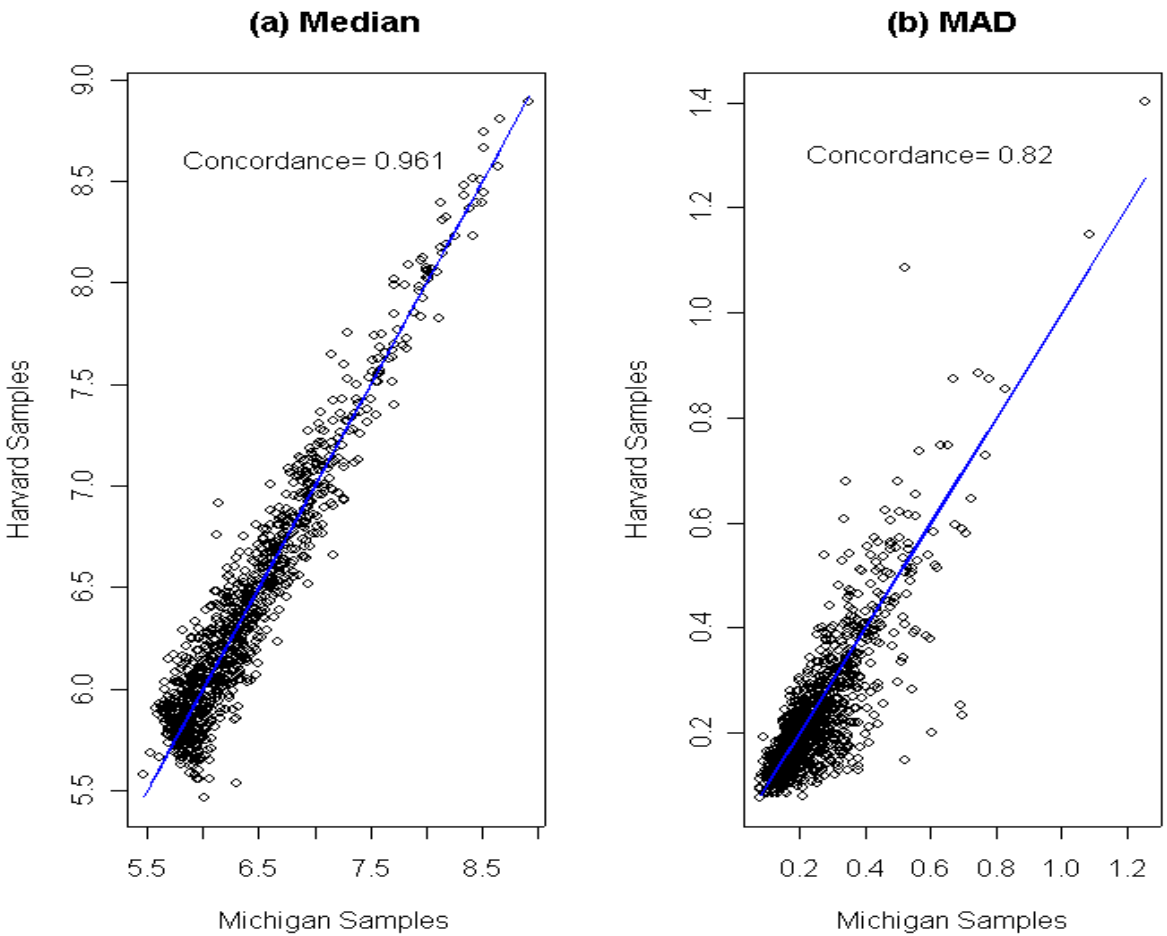
Quantification of Expression Levels

- Log-scale quantifications for each probeset obtained using PDNN model.
  - Uses Perfect Match (PM) probes only
  - Uses probe sequence info to predict patterns of specific and nonspecific hybridization intensities
  - Borrows strength across probe sets
- Shown to outperform dChip and MAS5.0
- See Zhang, et al. (2003) *Nature Biotech* for further details on method and comparison

Preprocessing

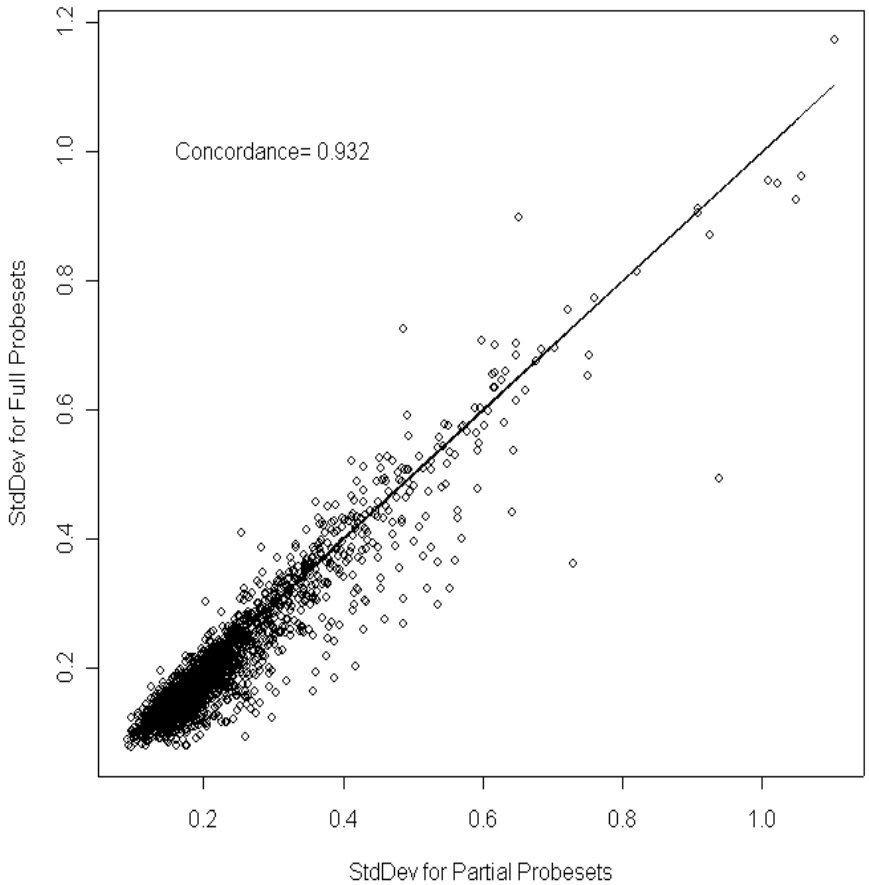
- Preprocessing steps:
  - Remove probesets with **smallest mean expression** levels across chips
  - **Normalize** log expression values within chips
  - Remove probesets with **smallest standard deviation** (<0.20) across chips
  - Remove probesets with **poor concordance** (<0.90) between partial and full probesets.
- **1036 probesets** remain after preprocessing

Assessing Our Method for Combining Information Across Chip Types



- "Partial Probeset" method appears to give **comparable expression levels** across chip types.

Assessing our Method for Combining Information across Chip Types



- Median "partial probeset" size is 7, vs. 16 or 20

  Loss of precision?
- No evidence of significant precision loss
- Also, relative ordering of samples well preserved (median r=0.95, using Spearman correlation)

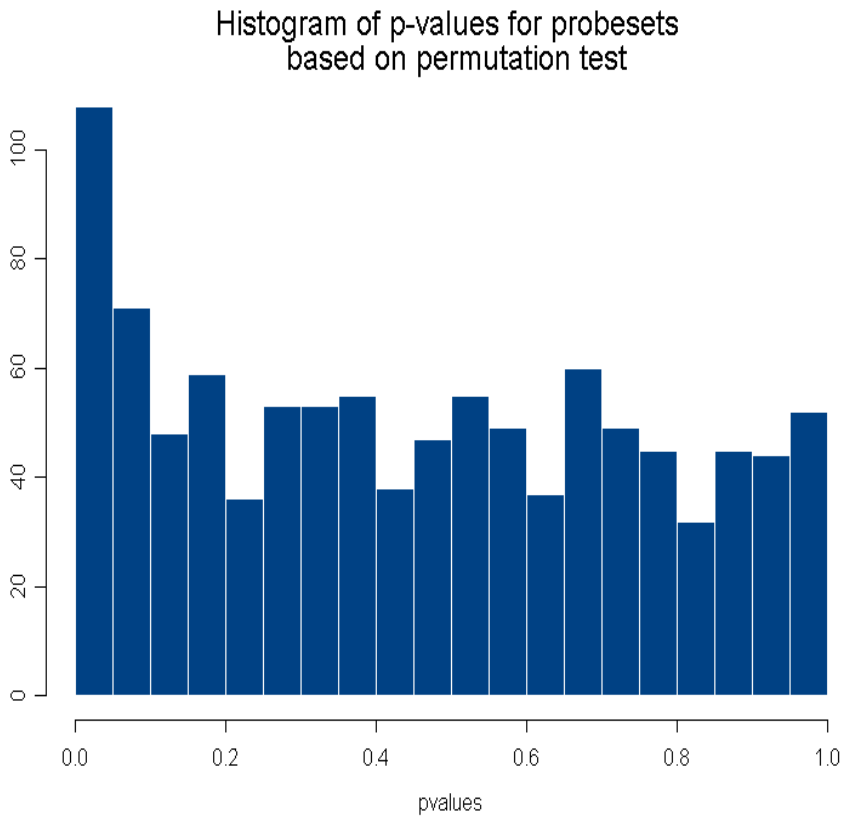
Identifying Prognostic Genes

- Series of 1036 **multivariable Cox models** fit to identify prognostic genes. Each model contained:
  - Study (Michigan=-1, Harvard=1).
  - Age (continuous factor).
  - Stage (early=0/late=1).
  - Probeset (log intensity value as continuous factor).
- Exact p-values for each probeset computed using **permutation approach**
- By using multivariate modeling, we search for genes offering prognostic information **beyond** clinical predictors

Identifying Prognostic Genes

- **BUM** method used to control **FDR<0.20**
  - Nonsignificant probesets → pvals Uniform
  - Significant probesets → more pvals near 0
  - Fit Beta-Uniform mixture to histogram of p-values
  - Model used to estimate FDR and get pval cutpoint
- Pounds and Morris, 2003 *Bioinformatics*

Results



- Histogram suggests there are some **significant probesets**
- FDR=0.20 corresponds pval cutoff of 0.0024
- **26** probesets flagged as significant

Selected Flagged Genes

Rank	Gene	β	p	Function
1	FCGRT	-2.07	<0.00001	Induced by IF-γ in treating SCLC
2	ENO2	1.46	0.00001	Marker of NSCLC
4	RRM1	1.81	0.00002	Linked to survival in NSCLC
8	CHKL	-1.43	0.00010	Marker of NSCLC
11	CPE	0.72	0.00031	Marker of SCLC
12	ADRBK1	-2.20	0.00044	Co-expressed with Cox-2 in lung ADC
16	CLU	-0.52	0.00109	Marker of SCLC
20	SEPW1	-1.29	0.00145	H202 cytotox. in NSCLC cell lines
21	FSCN1	0.66	0.00150	Marker of invasiveness in Stg 1 NSCLC
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Results

- Our gene list has almost no overlap with other publications of these data. Reasons:

1. We addressed a **different research question**
   - **Us**: ID Genes offering prognostic info beyond clinical
   - **Michigan**: Univariate Cox models fit; results used to construct dichotomous “risk index”
   - **Harvard**: Cluster analysis done; clusters linked to survival; found genes driving the clustering
2. Pooling across studies yielded **significant gains in statistical power**.
   - Most genes (17/26) in our study are not flagged if we analyze 2 data sets separately (i.e. no pooling)

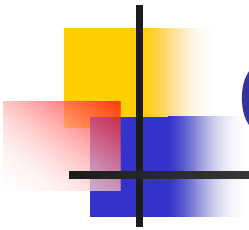
Conclusions

- New method for pooling info across studies using different versions of Affymetrix chips.
  - Recombine **matched probes** into **new probesets** using Unigene clusters.
  - Method appears to obtain **comparable** expression levels across chips without sacrificing much **precision** or significantly altering the **relative ordering** of the samples.

Conclusions

- Multivariate Cox models used to identify **new genes** offering prognostic information for lung adenocarcinoma patients.
  - Prognostic information **over and above** prognostic information provided by known clinical predictors.
  - Many of these genes seem **biologically interesting**.
  - It appears **increased statistical power** provided by the pooling helped in finding these new results.

- Pooling across studies:
  Great technical challenges, great gains to be realized
- CAMDA 2004 (http://www.camda.duke.edu/camda04)



Collaborators/Acknowledgements

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  - Guosheng Yin
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