The Armitage Lecture

Some statistics in bioinformatics

Patty Solomon

The University of Adelaide

Cambridge 15 November 2007
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'Short, but high quality'
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Phew!

Short, but high quality.
Peter’s pioneering 1960 book ‘Sequential Medical Trials’ was many, many years ahead of its time.
Motivation:
Post-WWII era of “controlled medical trials to compare the effectiveness of different therapeutic or prophylactic treatments”.

**PREFACE**

It is now widely accepted that the most reliable way to compare the effectiveness of alternative medical treatments is to carry out a controlled trial, in which the treatments are allocated at random. In recent years a number of these clinical trials have been conducted by sequential methods, and there appears to be a growing interest in this development. In a sequential analysis the observations are examined as they become available, and the total number of subjects to enter the trial is not predetermined, but depends on the accumulating results.

Sequential analysis has an immediate appeal in clinical research.

Peter’s book (and its 2nd edition in 1975) is a goldmine of information and good advice on experimental design (see next slide) ...
1.2 Comparative experimentation

- “If the same medical treatment is given to a number of patients with the same illness, their apparent responses will inevitably differ. Similar variation in response will appear if the treatment is given repeatedly to the same patient. Any attempt to compare the relative effectiveness of two or more treatments must therefore take into account the unpredictable variation in response from one occasion to another.”

- “The problem presents itself in almost every field of biological experimentation, for it is in the nature of biological material to show some degree of unpredictable variability.”

- “It has been generally recognized, since the work of R.A. (now Sir Ronald) Fisher in agricultural research in the 1920’s, that a valid comparison can be achieved only by some form of randomization ...”
Peter's early work on the comparison of survival curves stimulated much later research.
‘The comparison of survival curves’ compares the asymptotic relative efficiencies of four methods for comparing survival time distributions, when survival times are exponential:

- maximum likelihood
- the sign method*, in which individuals entering are paired and their times compared
- a comparison of proportions
- Kaplan-Meier.
THE COX REPORT

Short-term prediction of HIV infection and AIDS in England and Wales 1988

\[ S(t) = \begin{cases} 
(1 - \theta) & t = 0 \\
(1 - \theta)S(t) & t > 0 
\end{cases} \]

The overall distribution of survival time for UK AIDS patients, G.R. Reeves
The comparison of survival curves
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- Why? Because applications in medicine have significantly improved the human condition and saved many hundreds of thousands of lives.
Motivation

1. Introduction

The investigation reported in this paper arose out of a consideration of the possibility of using sequential methods in the design and analysis of clinical trials for treatments of chronic diseases in which the main criterion of success is length of survival after treatment. Professor A. Bradford Hill writes:

“In his discussion of Peter’s paper, Professor A. Bradford Hill writes:

“I am glad that Dr Armitage has turned his attention so helpfully to a problem that can be very troublesome in clinical medicine.”
Some Truths

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- HIV/AIDS pandemic
- challenges in early AZT trials
- method of backcalculation to reconstruct time-varying HIV infection incidence

As SIR model:

\[ a(t) = \int_0^t h(u)f(t - u) \, du \]
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Longini et al, Isaac Newton Institute, 1993
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Yeast metabolic interactions

Yeast protein-protein interactions
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III. Statistics is an *enabling discipline*.

It has its own internal dynamics and coherence, but good statistical analysis is the key to getting the best out of the new technologies.
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Why?

III. Statistics is an enabling discipline.

It has its own internal dynamics and coherence, but good statistical analysis is the key to getting the best out of the new technologies. We have by training the skills of experimental design, data analysis, synthesis and reasoning which are essential to bioinformatics and systems biology.
Some Truths

IV. The statistical sciences must themselves be strong to enable high-level collaborations with scientists from cognate disciplines.

V. “When we entered the era of high technology, we entered the era of mathematical technology”

Ad Hoc Committee on Resources for the Mathematical Sciences, US National Research Council, 1981
DNA makes RNA makes protein.

Mass spectrometry allows us to measure the protein complement (or subset thereof) of a set of cells.

There is a great deal of interest in discovering protein biomarkers in blood to identify cancer patients early on.
WHAT’S THE EXCITEMENT ABOUT?

- Profiles are being assessed using serum and urine, not tissue biopsies.
- Proteomic spectra are cheaper to run on a per unit basis than microarrays.
- Can run samples on large numbers of patients.
A MASS SPECTRUM OF HUMAN SERUM

What do the data look like?
Use of proteomic patterns in serum to identify ovarian cancer


Lancet, 359, 2002:572-7

http://home.ccr.cancer.gov.ncifdaproteomics
Use of proteomic patterns in serum to identify ovarian cancer


100 OVARIAN CANCER PATIENTS; 100 NORMAL CONTROLS; 16 PATIENTS WITH 'BENIGN DISEASE'

HTTP://HOME.CCR.CANCER.GOV.NCI:FDA:PROTEOMICS
Use of proteomic patterns in serum to identify ovarian cancer


- 100 ovarian cancer patients; 100 normal controls; 16 patients with 'benign disease'

- Used 50 cancer and 50 normal spectra to train a classifier and tested the algorithm on the remaining samples.

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Correctly classified 46/50 of the normal cases.

Correctly classified 16/16 of the benign disease as 'other'.

http://home.ccr.cancer.gov.ncifdaproteomics
*MUCH* EXCITEMENT ...

*SORACE & ZHAN BMC BIOINFORMATICS 2003; ZHANG ET AL CANCER RES 2004
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*SORACE & ZHAN BMC BIOINFORMATICS 2003; ZHANG ET AL CANCER RES 2004*
**All this (and more) strongly suggested a qualitative difference in how the samples were processed, and possibly not just a difference in the biology.**

**In January 2004, Correlogic, QuestDiagnostics and LabCorp announced plans to offer a ‘home brew’ test called OvaCheck: samples would be sent by clinicians for diagnosis.**

**Estimated market: 8 to 10 million women**

**Estimated cost: US$100-200 per test.**
IN AN ABORTIVE SECOND PAPER, CONDRADS ET AL.*

- Processed samples with their original SELDI technology and also with a higher resolution instrument (QSTAR). They added some QA/QC steps to remove bad spectra.

- Demonstrated 100% sensitivity and 100% specificity for identifying cancer from normal, and stated that this “emerging paradigm” is ready to go to a large clinical study.

SO WHAT WAS GOING ON?

* ENDOCRINE RELATED CANCER 11, 163-178, 2004
HERE ARE THEIR FIGURES 6A AND 7

COLOUR = DAY 1, 2, 3
What’s Going On?

Par III, Conrads et al., ERC (2004), Fig 6a & 7

*ALL* OF THE CONTROLS WERE RUN BEFORE *ALL* OF THE CANCERS.
THE MORAL OF THE STORY

☐ THERE IS NO WAY A WOMAN SHOULD BE TOLD SHE NEEDS AN OOPHORECTOMY BASED ON THESE TESTS!

☐ IN JUNE 2004, THE FDA RULLED THAT OVACHECK COULD NOT BE MADE AVAILABLE UNDER THE “HOME BREW” EXEMPTION, AS THE SOFTWARE PROGRAM WAS A ‘DEVICE’ THAT NEEDED TO BE MORE TIGHTLY REGULATED.

☐ IN SEPTEMBER 2006, THE FDA RELEASED DRAFT GUIDANCE ON ‘IN VITRO DIAGNOSTIC MULTIVARIATE INDEX ASSAYS’ (IVDMIAS).

☐ THESE RULES ARE BEING DEBATED EVEN NOW.
A story about how statistics is helping to discover genes involved in pluripotency

**Gene-profiling for a time course microarray experiment in stem cells**

**Pluripotency:** Refers to the potential of an early stem cell to develop into any type of mature cell, depending on environment.

Important in research ranging from organ transplants, the treatment of diabetes, to the treatment of spinal injuries.
Motivation for our research

Collaboration with Rathjen Lab, formerly of University of Adelaide.*

They have developed a mouse embryonic stem (ES) cell line for studying lineage specific differentiation.

Aim: to use microarrays to study changes in gene expression as a population of pluripotent ES cells are directed down the neuronal lineage via replacement of ES supporting media (LIF) with MEDII media.

“Omnibus” experiment.

* Australian Stem Cell Centre, ARC Special Research Centre for the Molecular Genetics of Development
Cell line model for studying directed differentiation of ES cells down neuronal lineage

4.5 dpc

\[ \text{Rex}^{1+}, \text{Oct}^{4+}, \text{Fgf}^{5-} \]

\[ \rightarrow \]

5.5 dpc

\[ \text{Rex}^{1^{-}}, \text{Oct}^{4+}, \text{Fgf}^{5+} \]

\[ \rightarrow \]

6.5 dpc

\[ \text{Rex}^{1^{-}}, \text{Oct}^{4^{-}}, \text{Sox}^{1^{-}} \]

\[ \rightarrow \]

Neural plate

\[ \text{Gbxb}^{2^{+}}, \text{Sox}^{1^{+}} \]

\[ \rightarrow \]

Neural tube

\[ \text{Gbxb}^{2^{-}}, \text{Sox}^{1^{+}} \]

\[ \rightarrow \]

ES

Day 0

\[ \rightarrow \]

EBM \(^3\)

Day 3

\[ \rightarrow \]

EBM \(^6\)

Day 6

\[ \rightarrow \]

EBM \(^9\)

Day 9
Aims, within resource constraints

- Study loss of pluripotence over time
- Identification of genes specific to each state
- Rank genes according to association with pluripotency.
- 20 hybridisations.
Design of the ES cell experiment

- CompuGen Mouse 22K long Oligo library
- 16 mRNA samples harvested on days 0, 3, 6, 9
- Passages p21 - p24 treated as biological replicates
The hypothetical profile for pluripotency

Log ratio with respect to Day 0 is plotted.
We want to identify genes with the pre-specified time-course profile.

Available methods typically not stringent enough - get part of profile, and spurious other parts. For example, Pareto optimization*; inner product of observed log ratios and a pre-specified profile**.

Require inferential procedures which accommodate testing hypotheses of equivalence of gene expression and hypotheses of differential gene expression simultaneously.

Gene profiling

We identify genes matching a pre-specified gene expression profile.

We treat the vector of true gene expression levels (for each gene) as a linear combination of linearly independent vectors, chosen to represent the pre-specified time profile.

The gene-profile model is fitted to the data, and the genes are ranked according to a suitable test statistic, incorporating equivalence and differential gene expression hypotheses.
Parameterisation for stem cell experiment

\[
\mu = 0 \begin{pmatrix} 1 \\ 1 \\ 1 \end{pmatrix} + 1 \begin{pmatrix} 1 \\ 1 \\ 0 \end{pmatrix} + 2 \begin{pmatrix} 1 \\ 1 \\ 0 \end{pmatrix} + 3 \begin{pmatrix} 1/2 \\ -1/2 \\ 0 \end{pmatrix}
\]

Pluripotent profile
With this choice of model, it follows that

\[
\begin{align*}
\gamma_0 &= \mu_9 \\
\gamma_1 &= \mu_0 + \frac{\mu_3}{2} - \mu_6 > 0 \\
\gamma_2 &= \mu_6 - \mu_9 > 0 \\
\gamma_3 &= \mu_0 - \mu_3 = 0
\end{align*}
\]

We want to actively \textit{demonstrate equivalence of gene expression} (on a gene-by-gene basis) on \textit{days 0 and 3}, not simply fail to find a\textit{ significant difference in the expression levels}. 
Null and alternative hypotheses:

\[ H_0 : |\gamma| \geq \epsilon, \quad \epsilon > 0 \]
\[ H_A : |\gamma| < \epsilon \]

Confidence interval inclusion: create \((L_\alpha(X), U_\alpha(X))\)

s.t. \( P(\gamma \in (L_\alpha(X), \infty)) = P(\gamma \in (-\infty, U_\alpha(X))) = 1 - \alpha \)

Reject null hypothesis in favour of equivalence iff

\( (L_\alpha(X), U_\alpha(X)) \subset (-\epsilon, \epsilon) \)
A snag ...

What do we mean by ‘equivalence’ of gene expression?

This is analogous to the “minimum clinically significant difference” of interest in a superiority trial or “tolerance limit” in a non-inferiority trial.

But, we have relatively little information on what it means to say genes are ‘equivalently expressed’, let alone their gene-specific variation, or their interactions with other genes. And we have lots of genes ...
Linear model for pluripotency

\( \mathbf{M} \) is the vector of observed log ratios

\[ \mathbf{M} = X^* \mu + E \]

we are measuring relative gene expression;
we estimate \( \gamma \) via least squares;

obtain an empirical Bayes estimate of \( \sigma^2 \).
Intersection-union test*

I-UT: 

\[ H_0 : \ (y_1 \leq 0) \cup (y_2 \leq 0) \cup (|y_3| \geq \epsilon), \quad \epsilon > 0 \]

\[ H_A : \ (y_1 > 0) \cap (y_2 > 0) \cap (|y_3| < \epsilon) \]

Rejection region (RR): intersection of separate rejection regions: \[ \alpha = \sup \alpha_y \]

Our aim is to rank the genes according to their match with the pre-specified profile: base on width of the largest c.i. contained within the RR.

Distance to the nearest boundary of the RR is calculated in standard errors of the estimate, larger values indicative of pluripotency.

*Berger, 1982
Pluripotency profile: fitted log ratios with respect to Day 0 for the ranked genes

### Ranked pluripotent-profile genes

<table>
<thead>
<tr>
<th>Gene Names</th>
<th>$\hat{c}_1$</th>
<th>$\hat{c}_2$</th>
<th>$\hat{c}_3$</th>
<th>U</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct4</td>
<td>0.48</td>
<td>2.77</td>
<td>0.20</td>
<td>2.53</td>
</tr>
<tr>
<td>Utf1</td>
<td>0.54</td>
<td>1.82</td>
<td>-0.42</td>
<td>2.46</td>
</tr>
<tr>
<td>Tdgf1</td>
<td>1.04</td>
<td>1.88</td>
<td>0.22</td>
<td>1.80</td>
</tr>
<tr>
<td>Slc35f2</td>
<td>0.32</td>
<td>1.69</td>
<td>-0.17</td>
<td>1.53</td>
</tr>
<tr>
<td>Trh</td>
<td>0.44</td>
<td>1.71</td>
<td>-0.69</td>
<td>1.50</td>
</tr>
<tr>
<td>Foxd3</td>
<td>0.14</td>
<td>1.79</td>
<td>-0.17</td>
<td>1.33</td>
</tr>
<tr>
<td>Musd1</td>
<td>0.15</td>
<td>2.00</td>
<td>-0.62</td>
<td>1.17</td>
</tr>
<tr>
<td>Skil</td>
<td>0.15</td>
<td>1.66</td>
<td>-0.83</td>
<td>1.16</td>
</tr>
<tr>
<td>Pou6f1</td>
<td>0.54</td>
<td>1.66</td>
<td>0.24</td>
<td>1.13</td>
</tr>
<tr>
<td>Par2</td>
<td>0.33</td>
<td>1.58</td>
<td>0.60</td>
<td>0.75</td>
</tr>
<tr>
<td>Nanog</td>
<td>0.31</td>
<td>1.99</td>
<td>0.88</td>
<td>0.69</td>
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<tr>
<td>Slc7a3</td>
<td>0.09</td>
<td>2.45</td>
<td>-0.58</td>
<td>0.67</td>
</tr>
<tr>
<td>Gng3</td>
<td>0.15</td>
<td>1.55</td>
<td>-0.42</td>
<td>0.33</td>
</tr>
<tr>
<td>Skil</td>
<td>0.23</td>
<td>1.54</td>
<td>-0.74</td>
<td>0.28</td>
</tr>
<tr>
<td>Rae-28</td>
<td>0.14</td>
<td>1.51</td>
<td>-0.29</td>
<td>0.08</td>
</tr>
</tbody>
</table>

**Protein interaction network, Wang et al, Nature 2006**
‘Sox2’ profile ranked genes

![Graph showing the 'Sox2' profile ranked genes over days with different log ratios.](image-url)
We note in passing that equivalence is not transitive, i.e., not invariant to re-parameterisation (but then nor are Wald tests, for example).

Gene profiling can be used for a variety of data types, e.g., microRNAs, single-channel gene expression data, ...

Straightforward to fit models in Limma, R and C.

Is proving valuable as a tool for exploring and building networks, finding patterns based on differences in treatment and control on a genome-wide scale.

Projects: role of transcription factor MYB in microRNAs; responses of Th1 and Th2 cells to IL4 and IL12.
Acknowledgements

Peter Armitage
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