Accumulation of genetic & epigenetic alterations: a key causal process between the environment and the occurrence of cancer.

Miquel Porta, MD, MPH, PhD
Institut Municipal d'Investigació Mèdica, Universitat Autònoma de Barcelona, and University of North Carolina at Chapel Hill.
www.imim.es/URECMC/eng

Accumulation of genetic & epigenetic alterations: is a key causal process between the environment and the occurrence of cancer.

Underestimation of environmental causes of the accumulation of genetic & epigenetic alterations in diseases of complex etiology is one of the features ideologically most characteristic, socially most relevant and, nonetheless, with a weaker scientific basis of contemporary biomedical research.


Epigenetic
Refers to mitotically or meiotically heritable changes in gene expression that do not involve a change in DNA sequence.

NATURE REVIEWS | GENETICS | APRIL 2007
EPIGENETICS:
heritable changes in gene expression that are not regulated by the DNA nucleotide sequence e.g., gene silencing by promoter hypermethylation or histone modification.

Impressive rediscovery of the influence of environmental agents on gene expression.

E.g.: Nickel, Cadmium, Arsenic: carcinogenicity also involves DNA hypermethylation and histone deacetylation, both of which contribute to heterochromatin condensation and the epigenetic silencing of some genes.

Impressive rediscovery of the influence of environmental agents on gene expression.
El archivo Audio de esta ponencia (38,8 MB) se encuentra en:
http://www.ipatimup-uecdc.com/porto_cancer_meeting/audio/miquel_porta.mp3
The history of cancer epigenetics

Andrew P. Feinberg and Benjamin Tycko

Nature Reviews | Cancer | February 2004


Epigenetic epidemiology

Eva Jablonka

Proteomics 2003, 3, 2402-2411
Mutation Research 558 (2004) 35-44

Proteomic analysis of plasma proteins of workers exposed to benzene


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http://www.ipatimup-uecdc.com/porto_cancer_meeting/audio/miquel_porta.mp3
Epigenetic epidemiology
Eva Jablonka

Almost by definition complex diseases depend on the intricate interplay of genetic and environmental factors that lead to changed epigenetic states.

Transgenerational epigenetic inheritance
the patterns of transmission of complex hereditary diseases may reflect the actions of non-mutagenic environmental agents and nutritional conditions on gene expression in ancestral generations, as well as the effects of the DNA that individuals actually inherited.

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http://www.ipatimup-uecdc.com/porto_cancer_meeting/audio/miquel_porta.mp3
Endocrine Disrupters Trigger Fertility Problems in Multiple Generations

“We’re mostly describing a new phenomenon,” acknowledges Skinner. But he is worried nonetheless. “The hazards of environmental toxins are much more pronounced than we realized,” he asserts.

...really?
Curves for each compound, by social class, geographic area .... .... ....

Curves for each compound, by social class, geographic area .... .... ....

Concentration in amniotic fluid of p,p’-DDT (ng/mL)

Percent of pregnancies

Cohort effects: what is their influence on the current burden of disease?

just a hypothesis...

public & private policies

1965

1985

2005

0.0 25

1945 65 75 85 95 2005 10 15

Exposure to Flame Retardants On the Rise

PCB

PCB2

PCDD/F

PCDF

Lipid-based concentration

14 June 2004, Vol 304, SCIENCE

PORTO CANCER MEETING - "Cancer etiology: bridging worlds"
IPATIMUP, Universidade do Porto (Portugal), 20-21 abril 2007
Miquel Porta -- página 13

El archivo Audio de esta ponencia (38,8 MB) se encuentra en:
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El archivo Audio de esta ponencia (38,8 MB) se encuentra en:
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Concentration in amniotic fluid of p,p'-DDT (ng/mL) by percent of pregnancies:

<table>
<thead>
<tr>
<th>Year</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1965</td>
<td>25</td>
</tr>
<tr>
<td>1985</td>
<td>20</td>
</tr>
<tr>
<td>2005</td>
<td>10</td>
</tr>
</tbody>
</table>

Cohort effects: what is their influence on the current burden of disease? *just a hypothesis...*

Molecular epidemiology, genetics & public health

On the lintel of his classic *The strategy of preventive medicine*, Geoffrey Rose (1926–1993) inscribed these words of Fyodor Dostoyevsky (1821–1881): “We are all responsible for all.” The idea that as citizens and societies we have shared, common responsibilities in front of threats to health is central to epidemiology, public health, even to clinical medicine... and to virtually all other professions and scientific disciplines. Why should it not also be relevant to urbanism, pedagogy, biology, or chemistry? It is of course also central to literature and most other forms of artistic expression.

Molecular epidemiology, genetics & public health

While these findings should not leave us indifferent, they are not particularly alarming. Mainly, because similar results would be obtained in most of us. But, would it not be more coherent to say that similar results would be obtained “in our populations”? Should we have the appropriate surveillance systems in place? Do we not know that there’s no effective individual escape from PTS? Then the path to follow is not to perform individual measurements of PTS, but population surveillance and control of PTS. Indeed, “Geoffrey Rose’s big idea” (changing the population distribution of a risk factor prevents more burden of disease than targeting people at high risk) is perfectly relevant to PTS—perhaps even more than to classic risk factors for chronic diseases. The only way forward is to shift the population distribution of PTS.

El archivo Audio de esta ponencia (38,8 MB) se encuentra en:
http://www.ipatimup-uecdc.com/porto_cancer_meeting/audio/miquel_porta.mp3
Persistent organic pollutants and the burden of diabetes

Studies from the USA\(^1\) have drawn attention to the possibility that persistent organic pollutants might contribute to cause diabetes.\(^2\)

Because they contaminate virtually all people, even if they confer only a low individual risk of diabetes, these pollutants might have a substantial overall population effect.\(^3\,4\)


El archivo Audio de esta ponencia (38,8 MB) se encuentra en:
http://www.ipatimup-uecdc.com/porto_cancer_meeting/audio/miquel_porta.mp3

Is there enough dialogue between epidemiology and the humanities?

Carcinogenesis is a multistage process driven by carcinogen-induced accumulation of genetic and epigenetic damage in susceptible cells that gain a selective growth advantage and undergo clonal expansion as the result of activation of protooncogenes and inactivation of tumor suppressor genes.

Therefore, the mutational spectra of chemical and physical carcinogens in critical genes are of interest to define endogenous and exogenous mutational mechanisms.


This essential evidence is often lacking for widely prevalent environmental chemical agents with well established toxic effects or with potential for interaction with gene products (e.g., with 'tumour promotion' properties or with potential for epigenetic effects).

**Molecular epidemiology, genetics & public health**

This is not only wrong for public health reasons, it is also weak on clinical and biological grounds.

However: vast majority of biomedical research is centred on genetic variants inhered and of low penetrance, e.g., in genes that confer 'susceptibility'.

And only a minority of research deals with:

a) population impact of reducing environ. exposures.

b) causes of acquired genetic alterations.

b1) envir. exposures as causes of acquired gene alters.

---

**Serum concentrations of p,p'-DDE (lipid-corrected, in ng/g) in the US general population**

<table>
<thead>
<tr>
<th>Geographical Group</th>
<th>Total, age 12 and older</th>
<th>10th</th>
<th>25th</th>
<th>50th</th>
<th>75th</th>
<th>90th</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total, age 12 and older</td>
<td>(214-266)</td>
<td>74.2</td>
<td>114</td>
<td>226</td>
<td>526</td>
<td>1120</td>
<td>1782</td>
</tr>
<tr>
<td>Age group</td>
<td>19-39 years</td>
<td>118</td>
<td>(40.9-90.6)</td>
<td>60.8</td>
<td>124</td>
<td>234</td>
<td>492</td>
</tr>
<tr>
<td>40-64 years</td>
<td>297</td>
<td>(55.9-165.4)</td>
<td>110</td>
<td>226</td>
<td>526</td>
<td>1120</td>
<td>1782</td>
</tr>
<tr>
<td>Gender</td>
<td>Males</td>
<td>240</td>
<td>(55.9-165.4)</td>
<td>110</td>
<td>226</td>
<td>526</td>
<td>1120</td>
</tr>
<tr>
<td>Females</td>
<td>370</td>
<td>(55.9-165.4)</td>
<td>110</td>
<td>226</td>
<td>526</td>
<td>1120</td>
<td>1782</td>
</tr>
<tr>
<td>Race/ethnicity</td>
<td>Mexican Americans</td>
<td>674</td>
<td>(193-706)</td>
<td>154</td>
<td>300</td>
<td>633</td>
<td>1330</td>
</tr>
<tr>
<td>Non-Hispanic blacks</td>
<td>296</td>
<td>(85.4-399)</td>
<td>113</td>
<td>255</td>
<td>542</td>
<td>1240</td>
<td>2480</td>
</tr>
<tr>
<td>Non-Hispanic whites</td>
<td>217</td>
<td>(85.4-399)</td>
<td>113</td>
<td>255</td>
<td>542</td>
<td>1240</td>
<td>2480</td>
</tr>
</tbody>
</table>

---

**Disease-related genetic alterations:**

- **Somatic or acquired**
- **Germinal or inherited**
  - of low penetrance
  - of high penetrance

---

Inherited low-penetrance variants and acquired genetic alterations do have common causal characteristics; e.g.:

A single mutation is never a sufficient cause of any of the most prevalent diseases, which are caused by survival, growth & selection of cell clones that have accumulated multiple alterations.

There often is a well established causal relationship between certain acquired mutations and many clinical diseases; e.g., in cancer, somatic mutations in the K-ras, p53 and other genes. Yet, causes of accumulated mutations are largely unknown.

There often is a well established causal relationship between certain environmental exposures and acquired genetic alterations; e.g., chemical carcinogenesis studies show that physical and chemical agents may activate oncogens, inactivate tumour suppressor & DNA repair genes...

---

The principle

**A:** One exposure, many diseases.

**B:** One disease, many genes of low penetrance + accumulation of genetic & epigenetic alterations.
A: 1 Exposure → Many Diseases

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Disease</th>
<th>Proportion attributable to exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tobacco smoke</td>
<td>Lung cancer</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td>Bladder cancer</td>
<td>70% (men) 30% (women)</td>
</tr>
<tr>
<td></td>
<td>Larynx cancer</td>
<td>90%</td>
</tr>
<tr>
<td></td>
<td>Coronary Heart D</td>
<td>12.5%</td>
</tr>
<tr>
<td></td>
<td>Chronic bronchitis</td>
<td>80%</td>
</tr>
</tbody>
</table>

B: One disease resulting from many low-penetrant genes

<table>
<thead>
<tr>
<th>Disease</th>
<th>Low-penetrant genes</th>
<th>Odds ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lung cancer</td>
<td>CYP1A1 Msp I</td>
<td>1.73 (Asian)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.04 (white)</td>
</tr>
<tr>
<td></td>
<td>CYP1A1 exon 7</td>
<td>2.25 (Asian)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1.30 (white)</td>
</tr>
<tr>
<td></td>
<td>CYP2D6</td>
<td>1.26</td>
</tr>
<tr>
<td></td>
<td>GSTM1</td>
<td>1.34</td>
</tr>
<tr>
<td>Bladder cancer</td>
<td>NAT-2 slow</td>
<td>1.37</td>
</tr>
<tr>
<td>Colon cancer</td>
<td>GSTM1</td>
<td>1.57</td>
</tr>
<tr>
<td></td>
<td>NAT-2 rapid</td>
<td>1.19</td>
</tr>
</tbody>
</table>

NNS: NUMBER NEEDED TO SCREEN to prevent 1 case of the disease.

A reasonable (low) NNS is attained only by screening for
- highly-penetrant mutations in high-risk families, not
- for such mutations in the general population, nor
- for low-penetrant polymorphisms.

• The relation between the frequency of a variant and its penetrance is almost inverse:
  the more penetrant (i.e., deleterious) a mutation, the less frequent in the population.

• The NNS to prevent 1 case is ↑↑
  - for low-penetrant polymorphisms and
  - for highly-penetrant mutations in the general population.
### Genetic Trait

<table>
<thead>
<tr>
<th>Penetrance</th>
<th>Frequency</th>
<th>Population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-penetrant</td>
<td>Common or Rare</td>
<td>General Population or Some Families</td>
</tr>
</tbody>
</table>

#### Las 3 situaciones reales

<table>
<thead>
<tr>
<th>Genetic Trait</th>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low-penetrant and Common in the General Population</td>
<td>High-penetrant and Rare in the General Population</td>
<td>High-penetrant and Common in Some Families</td>
<td></td>
</tr>
</tbody>
</table>

- **Prevalence of Carriers**
- **Identification leads to risk reduction of**
- **Lifetime risk of disease of carriers**
- **Absolute Risk Reduction**

**NNT = 1 / ARR**

**NNS = NNT / Pr Carriers**

### Number Needed to Screen to prevent 1 case

<table>
<thead>
<tr>
<th>A</th>
<th>B</th>
<th>C</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prevalence of Carriers</strong></td>
<td>13.8 per 100</td>
<td>90 per 100</td>
</tr>
<tr>
<td><strong>Identification leads to risk reduction of</strong></td>
<td>58 per 100</td>
<td>Same as A</td>
</tr>
<tr>
<td><strong>Lifetime risk of disease of carriers</strong></td>
<td>14 per 1,000</td>
<td>37 per 100</td>
</tr>
<tr>
<td><strong>Absolute Risk Reduction</strong></td>
<td>14 * 0.58 = 8</td>
<td>8 per 1,000</td>
</tr>
<tr>
<td><strong>NNT</strong></td>
<td>1,000 / 8 = 125</td>
<td>100 / 21.5 = 4.5</td>
</tr>
<tr>
<td><strong>NNS</strong></td>
<td>125 / 0.138 = 906</td>
<td>4.5 / 0.5 = 9</td>
</tr>
</tbody>
</table>


### Epidemiology • Volume 15, Number 1, January 2004

**Genetic Testing for Sale**

**Paolo Vines* and David C. Christiani†**

**SCIONA / BODY SHOP**

More expansive claims appear in advertisements for Sciona found in the European Body Shop stores: “Find out how your body copes with the following and what you need to eat to improve your body’s efficiency: Detoxifying—Is your body as efficient as it could be at removing toxins? Antioxidant Capacity—Does your body cope with free radicals as well as it should? Tissue Repair—Do you need to boost your vitamin intake to ensure effective tissue repair? Alcohol Metabolism—Can your body cope with alcohol consumption?”

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http://www.ipatimup-uecdc.com/porto_cancer_meeting/audio/miquel_porta.mp3
Genetic Epidemiology 4

Shaking the tree: mapping complex disease genes with linkage disequilibrium

Lyle J Palmer, Lon R Cardon

The genomics revolution has been accompanied by an unrealistic tendency to hyperbole. This has led to unrealistic expectations among clinicians and to cynicism and pessimism within the genetics community.

... or viceversa, which may be worse...

Lancet 2005; 366: 1223-34

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http://www.ipatimup-uecdc.com/porto_cancer_meeting/audio/miquel_porta.mp3
Bias as a threat to the validity of cancer molecular-marker research

Rules of evidence for cancer molecular-marker discovery and validation

There is considerable evidence that the translation rate of major basic science promises to clinical applications has been inefficient and disappointing. The deficiencies of translational science have often been proposed as an explanation for this failure. An alternative explanation is that until recently basic science advances have made oversimplified assumptions that have not matched the true etiological complexity of most common diseases; while clinical science has suffered from poor research practices, overt biases and conflicts of interest. The advent of molecular medicine and the rethinking of clinical science along the principles of evidence-based medicine provide a better environment where translational research may now materialize its goals. At the same time, priority issues need to be addressed in order to exploit the new opportunities. Translational research should focus on diseases with global impact. If true progress is to be made against human suffering, the health outcomes of interest for translational efforts need to be carefully defined and a balance must be struck between the subjective needs of healthcare consumers and objective health outcomes. Development of more simple, practical and safer interventions may be as important a target for translational research as the development of cures for diseases where no effective interventions are available at all. Moreover, while the role of the industry is catalytic in translating research advances to licensed interventions, academic independence needs to be sustained and strengthened at a global level. Conflicts of interest may stifle translational research efforts internationally. The profit motive is unlikely to be sufficient alone to advance biomedical research towards genuine progress.

Seminology, proteomics, and the early detection of symptomatic cancer

Exocrine pancreatic cancer: symptoms at presentation and their relation to tumour site and stage

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http://www.ipatimup-uecdc.com/porto_cancer_meeting/audio/miquel_porta.mp3
Incomplete overlapping of biological, clinical, and environmental information in molecular epidemiological studies: a variety of causes and a cascade of consequences

M. Porta, N. Malats, J. Vioque, A. Carrozzo, M. Soler, I. Reis, V. Barberà, D. Ayuso, F.X. Real

Cancer Epidemiology, Biomarkers & Prevention
Vol. 9, 1223–1222; November 2000

K-ras and p53 in Pancreatic Cancer: Association with Medical History, Histopathology, and Environmental Exposures in a Population-Based Study

out of >600 cases: 7 wt vs. 17 mutated

Attention to selection biases!

Generalizing Molecular Results Arising from Incomplete Biological Samples:

Expected Bias and Unexpected Findings

Miquel Porta, MD, MPH, PhD; Núria Malats, MD, PhD; Josep M. Corominas, MD, PhD; Josep L. Pinol, MD, PhD; and Francisco X. Real, MD, PhD; for the PAKRAS I Project Investigators

PURPOSE: In molecular epidemiology, obtaining biological samples for all subjects targeted for study is frequently hampered by ethical, logistical, and economic factors. The extent to which the incompleteness of biological samples could cause bias is rarely analyzed in depth. Here we report some expected biases and some unexpected findings during a study on mutations in the K-ras gene in exocrine pancreatic cancer

Attention to selection biases!

Disease-related genetic alterations:

- Somatic or acquired
- Other alterations
- Clinical disease

External factors

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Although a genetic profile for pancreatic cancer is emerging, many biological aspects of this disease are poorly understood. Indeed, fundamental questions regarding progenitor cell lineages, host stromal milieu, and the role of specific genetic alterations in tumor progression remain unresolved.


why...?


why...?

Why is not more research being done
– on ‘why’?
– on the causes of genetic alterations that have a well-established role in diseases of complex etiology?

why...?
MOLECULAR EPIDEMIOLOGY AND CANCER PREVENTION

Ras gene mutations in patients with Acute Myeloid Leukaemia and exposure to chemical agents

Emanuela Barletta, Giuseppe Gorini, Paolo Vineis et al.

In conclusion our data suggest that ras oncogene mutations might identify a group of leukaemia in people with previous X-ray/chemo-therapy or with exposure to chemical agents in the work environment.

Carcinogenesis vol.25 no.5 pp.749-755, 2004

Research on the causes of pancreatic cancer is a “natural meeting place” for basic science (knowledge on biological mechanisms), and epidemiology (knowledge for primary prevention).

Association between coffee drinking and K-ras mutations in exocrine pancreatic cancer

Miguel Porta, Núria Mata, Luisa Gomar, Attilio Cattaneo, Elda Rifi, Antonio Sales, Jose M Convit, Montserrat Andres, Francesc X Real for the PANKRAS II Study Group*

*Members of the Multicentre Prospective Study on the Role of the K-ras and other Genetic Alterations in the Diagnosis, Prognosis and Etiology of Pancreatic and Biliary Diseases (PANKRAS II). Study Group are listed in the appendix.

Correspondence to: Professor M Porta, Institut Municipal d’Investigació Mèdica, Universitat Autònoma de Barcelona, Carrer del Dr Aiguader 80, E-08003 Barcelona, Spain.

Conclusions—Pancreatic cancer cases without activating mutations in the K-ras gene had drank significantly less coffee than cases with a mutation, with a significant dose response relation the less they drank, the less likely their tumours were to harbour a mutation. In exocrine pancreatic cancer the K-ras gene may be activated less often among non-regular coffee drinkers than among regular drinkers. Caffeine, other coffee compounds or other factors with which coffee drinking is associated may modulate K-ras activation.

Carcinogenesis 1999;30:702-709

Research on the role of gene-environment interactions in the etiology of pancreatic cancer is generating basic knowledge on biological mechanisms, and for primary prevention.

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http://www.ipatimup-uecdc.com/porto_cancer_meeting/audio/miquel_porta.mp3
In a case-only design (...) the odds ratio in this table is taken as a measure of gene-environment interaction.
El archivo Audio de esta ponencia (38,8 MB) se encuentra en:
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These findings provided the first link between the most common oncogene mutation in human cancer and an environmental compound among humans living in normal conditions.

Now:
1. ORs for OC with 144 cases of EPC.
2. ORs for coffee adjusting by OC.
3. Adjusting by signs & symptoms.

The effect of DDT and coffee on the probability of a mutated (vs. wild-type) tumour.

<table>
<thead>
<tr>
<th>DDT Level</th>
<th>OR  (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;224 ng/g</td>
<td>1.0 (0.8-1.2)</td>
<td>0.61</td>
</tr>
<tr>
<td>225-614</td>
<td>3.1 (0.8-12.3)</td>
<td>0.111*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Coffee Level</th>
<th>OR  (95% CI)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non reg. drinkers</td>
<td>1.0 (0.4-1.4)</td>
<td>0.32</td>
</tr>
<tr>
<td>1-7 cups/week</td>
<td>3.8 (0.7-20.0)</td>
<td>0.111*</td>
</tr>
<tr>
<td>8-14 cups/week</td>
<td>2.4 (0.4-13.9)</td>
<td>0.32</td>
</tr>
<tr>
<td>≥15 cups/week</td>
<td>4.5 (0.9-22.7)</td>
<td>0.111*</td>
</tr>
</tbody>
</table>

ORs further adjusted by age, sex and constitutional syndrome. *Mantel’s test for linear trend.

The effect of DDT and coffee on the probability of a mutated (vs. wild-type) tumour.

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<tr>
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</tr>
<tr>
<td>8-14 cups/week</td>
<td>2.4 (0.4-13.9)</td>
<td>0.32</td>
</tr>
<tr>
<td>≥15 cups/week</td>
<td>4.5 (0.9-22.7)</td>
<td>0.111*</td>
</tr>
</tbody>
</table>

ORs further adjusted by age, sex and constitutional syndrome. *Mantel’s test for linear trend.
The effect of PCB 153 and coffee on the probability of a mutated (vs. wild-type) tumour.

<table>
<thead>
<tr>
<th>PCB 153 (ng/g)</th>
<th>OR</th>
<th>P-value (OR 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤187</td>
<td>1.0</td>
<td>0.003</td>
</tr>
<tr>
<td>188 – 313</td>
<td>3.9</td>
<td>(1.0-15.0)</td>
</tr>
<tr>
<td>&gt;313</td>
<td>11.4</td>
<td>(2.3-57.4)</td>
</tr>
</tbody>
</table>

Coffee

| Non reg. drinkers | 1.0 | 0.014* |
| 1-7 cups/week    | 4.1  | (0.8-20.0) |
| 8-14 cups/week   | 8.4  | (1.5-45.6) |
| ≥15 cups/week    | 8.0  | (1.5-41.6) |

ORs further adjusted by age, sex, and constitutional syndrome. *Mantel’s test for linear trend.

The (statistically) independent effect of DDT, PCB 153 and coffee on the probability of a mutated (vs. wild-type) tumour.

<table>
<thead>
<tr>
<th>p,p'-DDT (ng/g)</th>
<th>OR</th>
<th>P-value (OR 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤224</td>
<td>1.0</td>
<td>0.032</td>
</tr>
<tr>
<td>225 – 614</td>
<td>23.4</td>
<td>(2.0-267.3)</td>
</tr>
<tr>
<td>&gt;614</td>
<td>1.2</td>
<td>(0.2-6.3)</td>
</tr>
</tbody>
</table>

PCB 153

<table>
<thead>
<tr>
<th>PCB 153 (ng/g)</th>
<th>OR</th>
<th>P-value (OR 95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤187</td>
<td>1.0</td>
<td>0.029*</td>
</tr>
<tr>
<td>188 – 313</td>
<td>4.8</td>
<td>(0.8-28.4)</td>
</tr>
<tr>
<td>&gt;313</td>
<td>9.3</td>
<td>(1.3-66.6)</td>
</tr>
</tbody>
</table>

Coffee

| Non reg. drinkers | 1.0 | 0.048* |
| 1-7 cups/week    | 3.8  | (0.6-25.3) |
| 8-14 cups/week   | 4.9  | (0.6-39.2) |
| ≥15 cups/week    | 8.1  | (1.1-57.7) |

ORs further adjusted by age, sex, cholestatic syndrome and constitutional syndrome. *Mantel’s test for linear trend.

Some OC, as well as coffee, may have a co-causal role in the etiopathogenesis of K-ras mutated EPC through modulation of K-ras activation or persistence.

They might also have a similar role in other cancers in which K-ras mutations are also highly prevalent at diagnosis.
Conclusions / 2

Results are coherent with mechanistic hypotheses on an indirectly genotoxic role (perhaps, epigenetic) of some OC and of coffee.

The association was not indiscriminate with all OC: concentrations of HCB and β-HCH in cases were also high, and yet these OCs were not associated with an increased risk of mutation.

Results need to be refuted or replicated by other studies, which should also assess interactions among OC, and of OC with other environmental and genetic factors.

So what?

The high prevalence of these acquired genetic alterations [in K-ras] overall in human cancers and the generalized accumulation of OC in humans make it especially relevant to refute or to replicate the findings by other independent studies.

Disease-related genetic alterations:

- Somatic or acquired
- Other alterations
- . . .

Clinical disease

External factors

mechanisms
Somatic or acquired

External factors

Other alterations

... 

Clinical disease

Disease-related genetic alterations:

- Somatic or acquired
- Other alterations

mechanisms

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http://www.ipatimup-uecdc.com/porto_cancer_meeting/audio/miquel_porta.mp3
Disease-related genetic alterations:

The renowned “black box”

External factors

mechanisms

Clinical disease

Somatic or acquired

External factors

Other alterations

Clinical disease

Disease-related genetic alterations:

Generating knowledge:
1) on biological mechanisms, and
2) for primary prevention

mechanisms

there are good reasons to get inside the ‘box’.

Clinical disease

YES: difficult to detect: subtle, long-term effects. But...

NOT negligible:

number of individuals exposed to environmental chemical agents

www.cdc.gov/exposurereport

| Serum concentrations of p,p'-DDE (lipid-corrected, in ng/g) in the US general population |
|-----------------------------------------------|---|---|---|---|---|---|
|                                    | 10th | 25th | 50th | 75th | 90th | 95th |
| Total, age 12 and older            | 258  | 295  | 320  | 359  | 389  | 403  |
| Age group 50-64 years               | 65.4 | 74.1 | 82.7 | 94.7 | 109  | 125  |
|                                    | (57.4-73.3) | (66.0-83.7) | (78.7-90.7) | (90.4-105.0) | (102-118) | (116-137) |
| Females, 20 years and older        | 77.6 | 89.1 | 97.0 | 109  | 120  | 130  |
|                                    | (69.9-88.2) | (81.2-101.2) | (92.3-101.2) | (100-117) | (110-121) | (120-131) |
| Race/ethnicity, Mexican Americans  | 87.4 | 104 | 123 | 144 | 164 | 183 |
|                                    | (77.5-114) | (93.6-115) | (112-124) | (115-129) | (105-124) | (140-129) |
| Non-Hispanic blacks                | 79.5 | 91.2 | 100 | 117 | 136 | 150 |
|                                    | (66.9-91.3) | (83.6-109.5) | (94.1-104.1) | (106-121) | (107-129) | (124-127) |
| Non-Hispanic white                 | 73.5 | 87.1 | 97.9 | 110 | 126 | 135 |
|                                    | (62.4-88.8) | (76.2-108.2) | (90.5-105.6) | (100-120) | (101-122) | (119-120) |

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Somatic or acquired +

Other alterations +

... mechanisms

Clinical disease

External factors

there are good reasons to get inside the 'box'.

but does it work...?
Many companies and academic labs have joined the race to find so-called biomarkers, blood components like proteins or lipids that can signal disease.

Until now, said Dr. Howard Schulman, vice president of research and development at SurroMed, "biomarker discovery has relied on knowing everything possible about the disease," searching for proteins involved in the cause of the disease.

So, now, the search for biomarkers is shifting. Instead of trying to understand disease mechanisms, some companies are using new technology called proteomics to screen cells or blood rapidly, looking for proteins present in diseased people but not in healthy ones.

OvaCheck goes a step beyond that. It analyzes patterns made by all the proteins in the blood without even knowing what the proteins are.

There are thousands of data points (...). "We think now that there is an entire ocean of biomarkers that never before was known to exist," said Dr. Petricoin. He is co-director of the clinical proteomics program run by the F.D.A. and the National Cancer Institute with Dr. Lance A. Liotta, who helped develop the ovarian test.

Some experts say they would not trust a test in which the proteins being measured and their biological relationship to cancer are unknown. "If you don’t know what you’re measuring, it’s a dangerous black-box technology," said Dr. Eleftherios P. Diamandis, head of clinical biochemistry at Mount Sinai Hospital in Toronto.

But experts say OvaCheck must give virtually no false positives to make it useful for general screening. Fifteen women out of 100,000 get ovarian cancer each year, said Dr. Beth Y. Karlan, director of gynecologic oncology at Cedars-Sinai Medical Center in Los Angeles.

So if OvaCheck were used for yearly checks on the whole population, even a 1 percent rate of false positives would mean 1,000 false diagnoses for every 15 cases detected.

Back to the black-box?

Disease-related genetic alterations:
- Somatic or acquired
- External factors
- Often studies are restricted to inherited alterations in low penetrance genes, and researchers overlook how certain environmental processes interact biologically with the genetic material and cause mutations.
Often studies are restricted to inherited alterations in low penetrance genes, and researchers overlook how certain environmental processes interact biologically with the genetic material and cause mutations.

An excellent piece, except that...

Interactions among environmental factors and acquired genetic alterations are gene-environment interactions:

1) They are real physico-chemical interactions with DNA.

2) The environ. factor CAUSES the genetic alteration.

• Statistically, they are not interactions, they are a “main effect”. This is no scientific reason to elude them as interactions:
  It is well established that the biologic nature of the problem must guide its mathematical formulation (see Miettinen, Greenland, Pearce, Kleinbaum...)
Underestimation of environmental causes of the accumulation of genetic & epigenetic alterations in diseases of complex etiology is one of the features ideologically most characteristic, socially most relevant and, nonetheless, with a weaker scientific basis of contemporary biomedical research.

Molecular epidemiology, genetics & public health

This is not only wrong for public health, it is also weak on clinical and biological grounds.

However: vast majority of research is centred on genetic variants inherited and of low penetrance, e.g., in genes that confer 'susceptibility'.

And only a minority of research deals with:

a) population impact of reducing environmental exposures.
b) causes of acquired genetic alterations.
c) how environ. exposures cause acquired gene alterations.

9 Limitations of studies on inherited genetic variants of low penetrance:

Limitations – inherent to the nature of studies or – related to their uses in public spaces

Vol. 11, 1544-1549, December 2002 Cancer Epidemiology, Biomarkers & Prevention

Commentary

Why Have We Failed to Find the Low Penetrance Genetic Constituents of Common Cancers?

Neil E. Caporaso
Genetic Epidemiology Branch, National Cancer Institute, Rockville, Maryland

Harri Vainio
Scandinavian Journal of Work Environment & Health
9 Limitations of studies on inherited genetic variants of low penetrance:

1. The variant is inherited and thus non-modifiable.
2. The variant has no clinical impact if there is no exposure.
3. The socialisation of studies attenuates or silences the effect of the environmental exposure.
4. The biologic & clinical effect is determined by different variants.
5. The diversity of mechanisms supports global analyses of the haplotypes.
6. Analyses of single exposures are seldom justified: mixtures of exposures are the rule.
7. The genetic variant has a weak influence on the clinical phenotype (low OR; lifetime Risk Difference is often unknown).
8. A given polymorphism can have + and – effects in different tissues.
9. Low biologic and epidemiologic plausibility of calculations on the population attributable risk for a single given genotype.

9 Limitations of studies on inherited genetic variants of low penetrance:

9. Low biologic and epidemiologic plausibility of calculations on the population attributable risk for a single given genotype.
9 Limitations of studies on inherited genetic variants of low penetrance:

9. Low biologic and epidemiologic coherence of many studies (molecular epidemiol. and molecular biology).

Other essential problems (methodological, epistemological & ontological):

- **Underestimation of causal complexity.**
  i.e., common underestimation of the complexity of gene-environment interactions:
  - wide changes in fluxes of exposure and excretion during lifetime or causally relevant ‘exposure-window’,
  - different effects at different doses for same agent (saturation and hormesis...),
  - dynamics of gene-gene and exposure-exposure (mixtures) interactions...

- **Oversimplification in the design of the “object of the study”**
  (Miettinen; Bolúmar & Porta, Eur J Epidemiol 2004)
  e.g., genotypes are not static “exposures”, but dynamic sources of proteins;
  “robustness” and “redundancy”...
  1 gene → > 1 protein....
  1 genotype → > 1 phenotype...

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It is unimportant that the language sounds too ecclesiastic to us. We simply find such propositions at odds with much of the contemporary scientific world: wide open, transdisciplinary—much more creative, relevant, efficient, and interesting because of the porousness, flexibility and adaptability of the disciplines than because of the putative higher mission of their clerics and disciples.

It is almost certain that epidemiology would benefit from a stronger philosophical base, including epistemology and ontology. What we are not sure of is whether we are losing a great expert in scientific methods while really gaining a philosopher of epidemiology. It would’ve been too easy to title this essay ‘Quo vadis, Olli Miettinen?’

Other essential problems (methodological, epistemological & ontological):

- Underestimation of causal complexity.

  Underestimation of time dynamics or time-related events.

  Studies are much too cross-sectional.

  e.g., too often there is just 1 single measurement of the exposure or of the “biomarker”, no consideration of intermediate processes, compensatory mechanisms, reversible effects...
Other essential problems (methodological, epistemological & ontological):

- Underestimation of causal complexity.
  - We need studies with a much stronger biological rationale
  - with a much stronger clinical rationale
  - truly longitudinal
  - with repeated measures for each individual
  - with more public health “sense & sensitivity”.

THANK YOU FOR YOUR ATTENTION