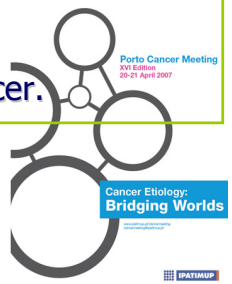
Accumulation
of genetic & epigenetic alterations:
a key causal process
between the environment
and the occurrence of cancer.




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



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.

*Gac Sanit. 2005;19(4):273-6*



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

EDITORIAL

La acumulación de alteraciones genéticas y epigenéticas:
un proceso causal clave entre el medio ambiente
y las enfermedades de etiología compleja

*Gac Sanit. 2005;19(4):273-6*

(Accumulation of genetic and epigenetic alterations: a key causal process between the environment and diseases of complex etiology)



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

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

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.

*Andreas Luch*
FEBRUARY 2005
NATURE REVIEWS | CANCER
NATURE AND NURTURE – LESSONS FROM CHEMICAL CARCINOGENESIS



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.

*Andreas Luch*
FEBRUARY 2005
NATURE REVIEWS | CANCER
NATURE AND NURTURE – LESSONS FROM CHEMICAL CARCINOGENESIS



ELSEVIER
Mutation Research 533 (2003) 107–120
MR Fundamental and Molecular Mechanisms of Mutagenesis

Review

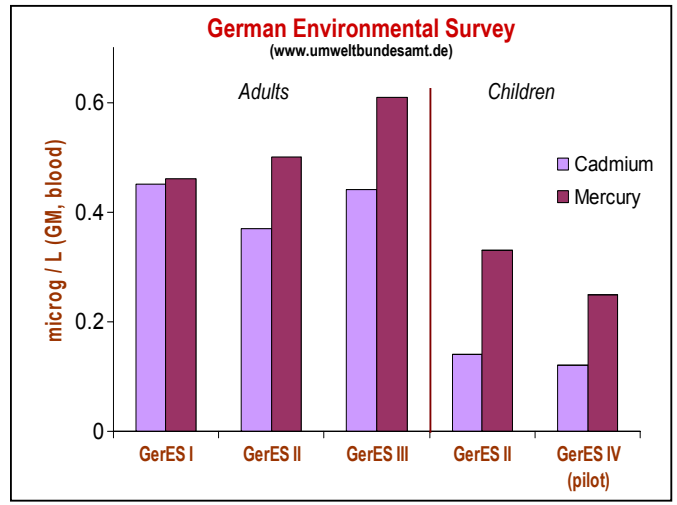
# Cadmium carcinogenesis

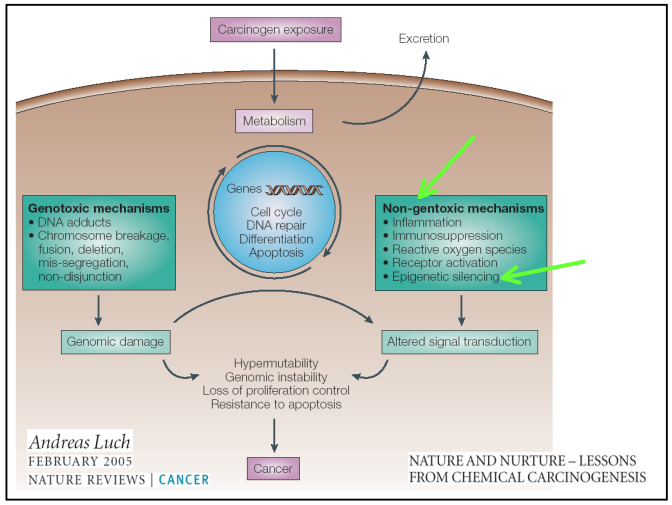
Michael P. Waalkes*

Cadmium compounds are classified as
human carcinogens by several regulatory agencies.

Most studies indicate cadmium is poorly mutagenic
and probably acts through indirect or epigenetic mechanisms,
potentially including aberrant activation of oncogenes
and suppression of apoptosis.








PORTO CANCER MEETING - "Cancer etiology: bridging worlds"
IPATIMUP, Universidade do Porto (Portugal), 20-21 abril 2007
Miquel Porta -- página 5

**Cell Cycle Control, Checkpoint Mechanisms, and Genotoxic Stress**

Rodney E. Shackelford,[1] William K. Kaufmann,[2] and Richard S. Paules[1,2]

Environmental Health Perspectives ▪ Vol 107, Supplement 1 ▪ February 1999

One important and interesting area for future study is the impact of nongenotoxic chemicals on cell cycle checkpoint function. A number of chemicals found in the environment, compounds such as benzene and 1,4-dioxane, fail to show mutagenic properties as measured in Salmonella mutagenesis assays, yet have the ability to induce tumors in rodents. The mechanism of induction of neoplasia by these environmental chemicals and their effects on cell cycle checkpoint function are not yet clearly understood. It is possible for example, that a nongenotoxic environmental carcinogen may function by ablating some aspects of cell cycle checkpoint function, perhaps leading to genetic instability or heritable alterations of the genome.

Environmental sources of genotoxic stress. Humans come into daily contact with an enormous number of DNA-damaging agents. Therefore, it is not surprising that elaborate molecular regulatory systems exist to maintain cellular genomic integrity. Genotoxic substances may come from both endogenous and exogenous sources.

Understanding the role of cell cycle checkpoint responses to environmental exposures promises to aid in the development of more efficacious approaches to disease prevention.

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PORTO CANCER MEETING - "Cancer etiology: bridging worlds"
IPATIMUP, Universidade do Porto (Portugal), 20-21 abril 2007
Miquel Porta -- página 6

FOCUS ON EPIGENETICS

REVIEWS

# Environmental epigenomics and disease susceptibility

*Randy L. Jirtle* and Michael K. Skinner‡*

Abstract | Epidemiological evidence increasingly suggests that environmental exposures early in development have a role in susceptibility to disease in later life. In addition, some of these environmental effects seem to be passed on through subsequent generations. Epigenetic modifications provide a plausible link between the environment and alterations in gene expression that might lead to disease phenotypes. An increasing body of evidence from animal studies supports the role of environmental epigenetics in disease susceptibility. Furthermore, recent studies have demonstrated for the first time that heritable environmentally induced epigenetic modifications underlie reversible transgenerational alterations in phenotype. Methods are now becoming available to investigate the relevance of these phenomena to human disease.

NATURE REVIEWS | GENETICS | APRIL 2007

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# The history of cancer epigenetics

*Andrew P. Feinberg and Benjamin Tycko*

NATURE REVIEWS | CANCER | FEBRUARY 2004

*International Journal of Epidemiology* 2004;**33**:929–935

## 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

Won-A Joo[a], Donggeun Sul[b], Do-Youn Lee[a], Eunil Lee[b], Chan-Wha Kim[a,*]



The history of cancer epigenetics

*Andrew P. Feinberg and Benjamin Tycko*

Box 1 | **The three main types of epigenetic information**

Cytosine DNA methylation is a covalent modification of DNA, in which a methyl group is transferred from *S*-adenosylmethionine to the C-5 position of cytosine by a family of cytosine (DNA-5)-methyltransferases. DNA methylation occurs almost exclusively at CpG nucleotides and has an important contributing role in the regulation of gene expression and the silencing of repeat elements in the genome.

Genomic imprinting is parent-of-origin-specific allele silencing, or relative silencing of one parental allele compared with the other parental allele. It is maintained, in part, by differentially methylated regions within or near imprinted genes, and it is normally reprogrammed in the germline.

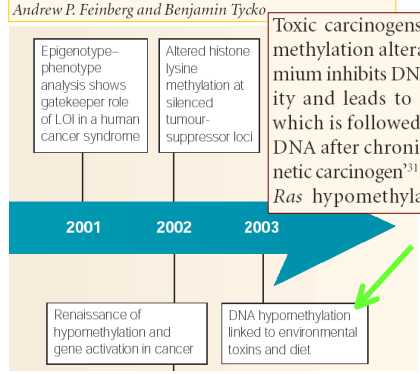
Histone modifications — including acetylation, methylation and phosphorylation — are important in transcriptional regulation and many are stably maintained during cell division, although the mechanism for this epigenetic inheritance is not yet well understood. Proteins that mediate these modifications are often associated within the same complexes as those that regulate DNA methylation.

NATURE REVIEWS | CANCER | FEBRUARY 2004



The history of cancer epigenetics

*Andrew P. Feinberg and Benjamin Tycko*




Toxic carcinogens might also act through methylation alterations. For example, cadmium inhibits DNA methyltransferase activity and leads to acute hypomethylation, which is followed by hypermethylation of DNA after chronic exposure to this 'epigenetic carcinogen'[31]. Similarly, arsenic induces *Ras* hypomethylation in mice[32]. Finally,

NATURE REVIEWS | CANCER | FEBRUARY 2004

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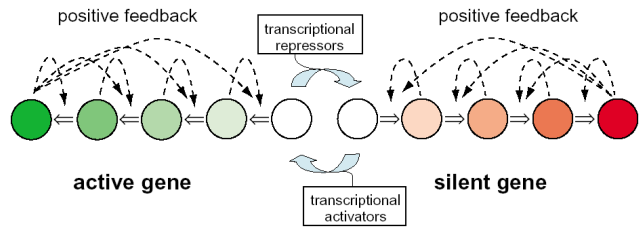
Miquel Porta -- página 8




Rudolf Jaenisch[1] & Adrian Bird[2]

## Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals

**nature genetics supplement • volume 33 • march 2003**

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

*International Journal of Epidemiology* 2004;**33**:929–935

# 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.



*International Journal of Epidemiology* 2004;**33**:929–935

# Epigenetic epidemiology

Eva Jablonka

Effective disease prevention and treatment will have to overcome the inertia caused by the persistence of epigenetic effects that are the result of exposure to toxicants and pollutants in earlier generations: removing present offending environmental factors may not be enough—it may need active and specific compensation for past epigenetic programming.[49]



www.sciencemag.org SCIENCE VOL 308 3 JUNE 2005

# Epigenetic Transgenerational Actions of Endocrine Disruptors and Male Fertility

**Matthew D. Anway, Andrea S. Cupp,* Mehmet Uzumcu,† Michael K. Skinner‡**

Transgenerational effects of environmental toxins require either a chromosomal or epigenetic alteration in the germ line. Transient exposure of a gestating female rat during the period of gonadal sex determination to the endocrine disruptors vinclozolin (an antiandrogenic compound) or methoxychlor (an estrogenic compound) induced an adult phenotype in the $F_1$ generation of decreased spermatogenic capacity (cell number and viability) and increased incidence of male infertility. These effects were transferred through the male germ line to nearly all males of all subsequent generations examined (that is, $F_1$ to $F_4$). The effects on reproduction correlate with altered DNA methylation patterns in the germ line. The ability of an environmental factor (for example, endocrine disruptor) to reprogram the germ line and to promote a transgenerational disease state has significant implications for evolutionary biology and disease etiology.

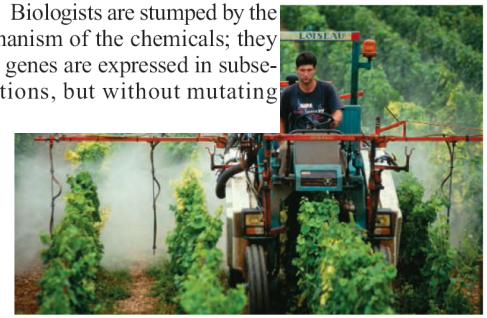


www.sciencemag.org SCIENCE VOL 308 3 JUNE 2005

DEVELOPMENTAL BIOLOGY

## Endocrine Disrupters Trigger Fertility Problems in Multiple Generations

Biologists are stumped by the apparent mechanism of the chemicals; they may alter how genes are expressed in subsequent generations, but without mutating DNA.



**Unfertile ground.** The fungicide vinclozolin, which is sprayed on vineyards like these, can cause fertility problems in male offspring of exposed rats.

www.sciencemag.org SCIENCE VOL 308 3 JUNE 2005

DEVELOPMENTAL BIOLOGY

## 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?






American Journal of Epidemiology


## In Utero Exposure to Background Concentrations of DDT and Cognitive Functioning among Preschoolers

Núria Ribas-Fitó[1], Maties Torrent[2], Daniel Carrizo[3], Laura Muñoz-Ortiz[1], Jordi Júlvez[1], Joan O. Grimalt[3], and Jordi Sunyer[1]

*p,p'*-DDT (bis[*p*-chlorophenyl]-1,1,1-trichloroethane) is a persistent organochlorine compound that has been used worldwide as an insecticide. The authors evaluated the association of cord serum levels of DDT and its metabolite, 2,2-bis(*p*-chlorophenyl)-1,1-dichloroethylene (DDE), with neurodevelopment at age 4 years. Two birth cohorts in Ribera d'Ebre and Menorca (Spain) were recruited between 1997 and 1999 ($n = 475$). Infants were assessed at age 4 years by using the McCarthy Scales of Children's Abilities. Organochlorine compounds were measured in cord serum. Children's diet and parental sociodemographic information was obtained through questionnaire. Results showed that DDT cord serum concentration at birth was inversely associated with verbal, memory, quantitative, and perceptual-performance skills at age 4 years. Children whose DDT concentrations in cord serum were >0.20 ng/ml had mean decreases of 7.86 (standard error, 3.21) points in the verbal scale and 10.86 (standard error, 4.33) points in the memory scale when compared with children whose concentrations were <0.05 ng/ml. These associations were stronger among girls. Prenatal exposure to background, low-level concentrations of DDT was associated with a decrease in preschoolers' cognitive skills. These results should be considered when evaluating the risk and benefits of spraying DDT during antimalaria and other disease-vector campaigns.



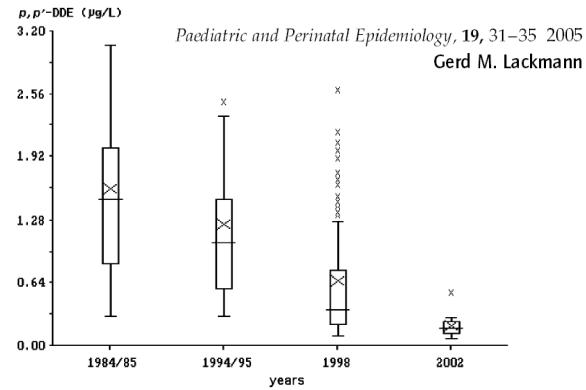
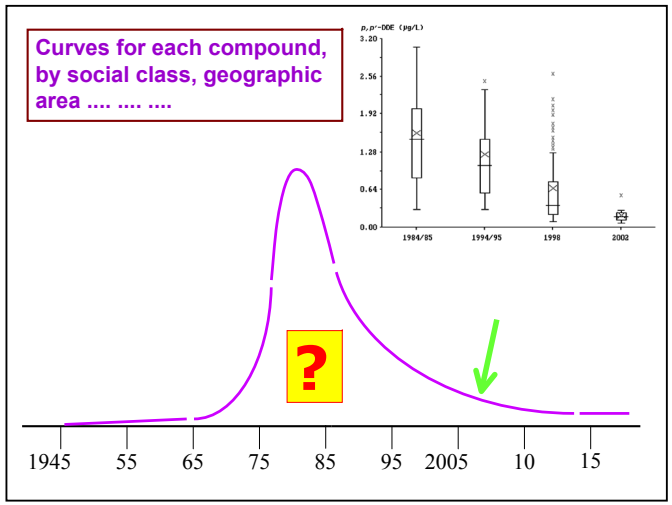
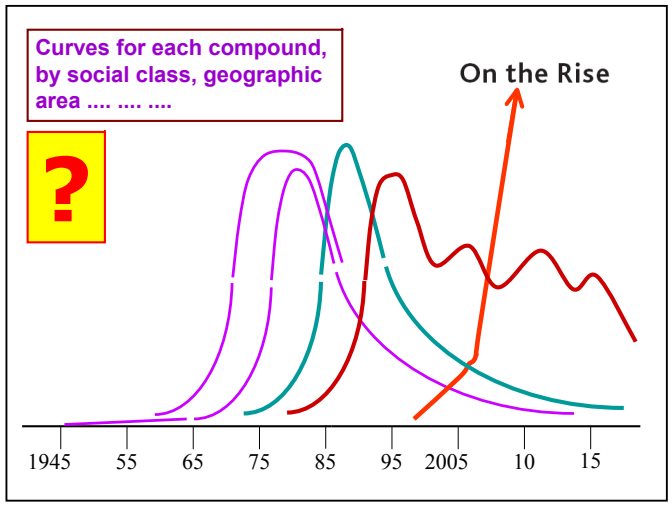



**Figure 1.** Box & Whisker-diagram of *p,p'*-DDE concentrations (µg/L) in full-term German neonates from the mid-1980s to 2002.



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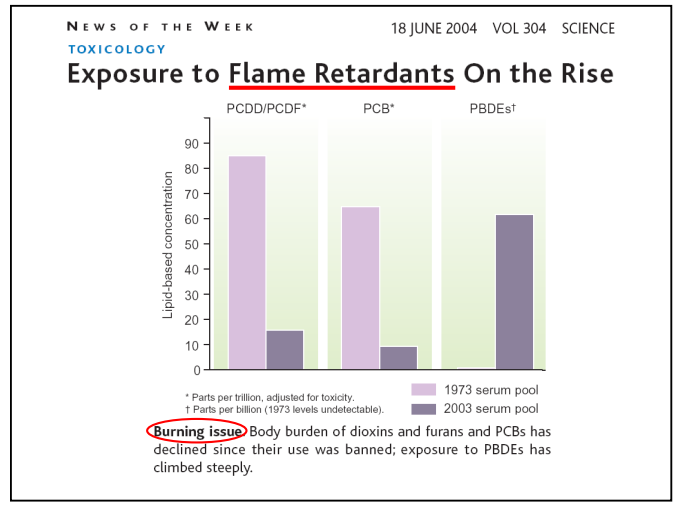




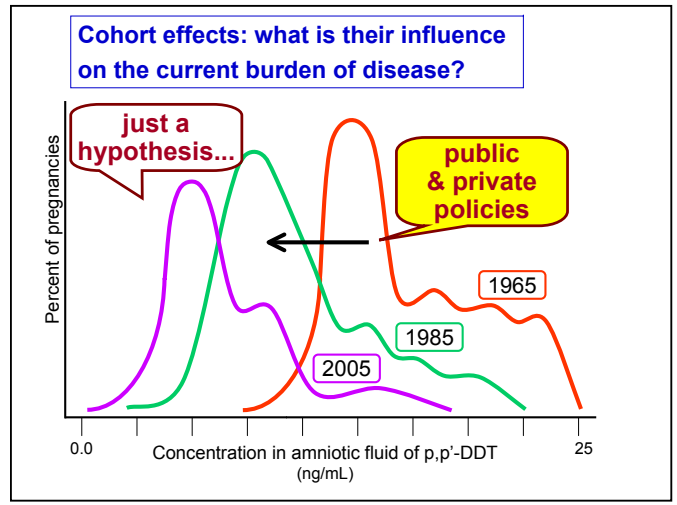


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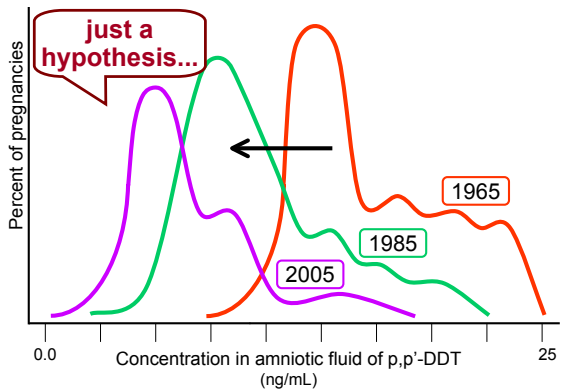
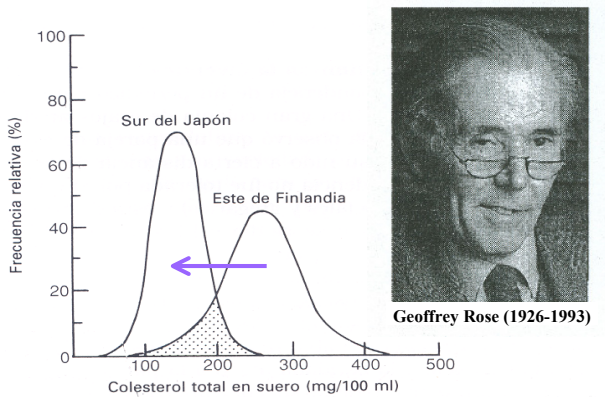
**Cohort effects: what is their influence on the current burden of disease?**







Geoffrey Rose (1926-1993)

Diferentes distribuciones de las cifras de colesterol en suero en el sur del Japón y el este de Finlandia.

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*J Epidemiol Community Health* 2004;**58**:534–535.

Persistent toxic substances: exposed individuals and exposed populations

## Molecular epidemiology, genetics & public health

On the lintel of his classic *The strategy of preventive medicine*,[1] 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.



*J Epidemiol Community Health* 2004;**58**:534–535.

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",[1 22] 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 Roses's big idea"[23] (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.[4–8] The only way forward is to shift the population distribution of PTS.

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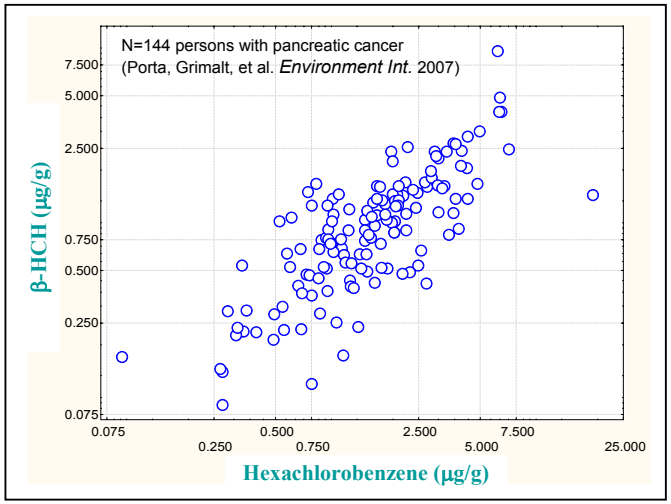




# THE LANCET

**Vol 368 August 12, 2006** *Miquel Porta*

**Persistent organic pollutants and the burden of diabetes**

Studies from the USA[1,2] have drawn attention to the possibility that persistent organic pollutants might contribute to cause diabetes.[3-6]

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.[10]



# THE LANCET

**Vol 368 August 12, 2006** *Miquel Porta*

**Persistent organic pollutants and the burden of diabetes**

When assessing the mechanisms linking diet, fat intake, obesity, and diabetes, persistent organic pollutants should also be considered. We need a better understanding of the burden of diabetes that these pollutants might contribute to cause.



JAUME PLENSA Songs and Shadows

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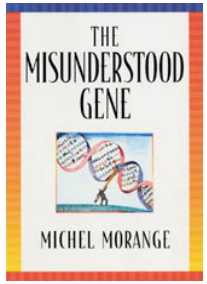
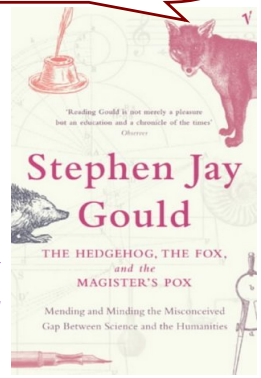



HARVARD UNIVERSITY PRESS
2001

**Is there enough dialogue between epidemiology and the humanities?**




***The hedgehog, the fox and the magister's pox. Mending and minding the misconceived gap between science and the humanities.***
London: Vintage, 2004.



*International Journal of Epidemiology* 2003;**32**:29–31

## The genome sequence is a jazz score

Miquel Porta

*It is not possible to do the work of science without using a language that is filled with metaphors.*

In: The Triple Helix (2000) Richard C Lewontin

The main purpose of this paper is to suggest a metaphor—among many possibly valid and evocative—for the role of genes in complex chronic diseases. It is based on the inherent role of host-environmental interactions on the expression of low-penetrant genes. The relationship between an individual's genetic makeup and its phenotypic expression can be likened to the relationship between a jazz score and the performed music.



Social Science & Medicine 56 (2003)

## Locating gene–environment interaction: at the intersections of genetics and public health

Sara Shostak*

the increasing focus on gene–environment interaction directs scientific, biomedical, and public health attention both *inward*, to the gene/genome, and *outward*, to particular places. In so doing, studies of gene–environment interaction create a challenging and productive tension—at the same time that bodies are being geneticized (Am. J. Law Med. 17 (1992) 15), they also are emphatically emplaced, located where social and cultural practices come to matter. This tension, this simultaneous movement outward and inward, towards the gene and towards the environment, into

Social Science & Medicine 56 (2003)

## The social life of genes privacy, property and the new genetics

Margaret Everett*

El archivo Audio de esta ponencia (38,8 MB) se encuentra en:
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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**.

Harris CC. Cancer Research, 1991.

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

*Proteomics* 2003, *3*, 2402–2411

Mutation Research 558 (2004) 35–44

# Proteomic analysis of plasma proteins of workers exposed to benzene

Won-A Joo [a], Donggeun Sul [b], Do-Youn Lee [a], Eunil Lee [b], Chan-Wha Kim [a,*]

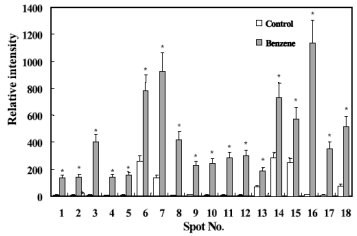



Fig. 2. Relative intensity of up-regulated proteins in benzene-exposed workers. The spot image was analyzed using the ImageMaster 2D Elite Software. The volume of the spots was calculated by total spot normalization, and each spot quantity was expressed as relative intensity. Each bar represents the mean ± S.D. of relative intensity of each spots. Significant differences were found between unexposed individual and benzene exposure based on a two-tailed Student's test ($P < 0.01$).





World Health Organization

www.elisabettafarina.com

http://www.who.int/reproductive-health/artforhealth/gallery.htm

www.thelancet.com Vol 368 December 16, 2006



## Molecular epidemiology, genetics & public health

**1 Population distribution of environmental exposures.**
**Relative risks for exposure - disease.**
**Interactions among exposures.**
**Population attributable risks / Burden of illness caused by environmental exposures.**

**2 Population distribution of genetic alterations (acquired) and genetic variants (inherited).**
**Burden of disease caused by genetic alterations.**

**3 Population distribution of gene-gene and gene-environment interactions.**
**Clinical effects (caused by interactions).**
**Burden of illness (caused by interactions).**

Khoury M et al. Oxford University Press, 2000.



## Molecular epidemiology, genetics & public health

**1 Population distribution of environmental exposures.**
**Relative risks for exposure - disease.**
**Interactions among exposures.**
**Population attributable risks / Burden of illness caused by environmental exposures.**

**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).**

Khoury M et al. Oxford University Press, 2000.

www.cdc.gov/exposurereport

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

| | Geometric mean (95% conf. Interval) | Selected percentiles (95% confidence interval) | | | | | | Sample size |
|---|---|---|---|---|---|---|---|---|
| | | 10th | 25th | 50th | 75th | 90th | 95th | |
| Total, age 12 and older | 260 (234-289) | 74.2 (66.1-84.2) | 114 (99.8-129) | 226 (191-267) | 538 (485-609) | 1120 (991-1290) | 1780 (1520-2230) | 1964 |
| Age group | | | | | | | | |
| 12-19 years | 118 (101-137) | 45.9 (34.9-56.6) | 69.8 (59.2-80.4) | 108 (90.6-132) | 185 (141-233) | 343 (255-479) | 528 (364-644) | 686 |
| 20 years and older | 297 (267-330) | 86.0 (75.2-96.7) | 130 (115-150) | 269 (229-303) | 626 (538-697) | 1250 (1100-1420) | 1990 (1570-2510) | 1278 |
| Gender | | | | | | | | |
| Males | 249 (221-281) | 77.6 (68.6-88.2) | 119 (101-133) | 222 (182-266) | 489 (383-570) | 985 (756-1130) | 1350 (1190-1610) | 937 |
| Females | 270 (241-302) | 68.9 (55.1-82.5) | 112 (96.0-129) | 228 (191-286) | 604 (516-697) | 1320 (1100-1600) | 2150 (1650-2750) | 1027 |
| Race/ethnicity | | | | | | | | |
| Mexican Americans | 674 (572-795) | 154 (133-214) | 300 (252-370) | 623 (505-750) | 1350 (1090-1660) | 3090 (2100-4610) | 4940 (3280-7810) | 657 |
| Non-Hispanic blacks | 295 (253-344) | 62.2 (56.9-80.5) | 113 (98.3-128) | 203 (164-253) | 452 (392-571) | 1340 (974-1910) | 2160 (1470-4010) | 416 |
| Non-Hispanic whites | 217 (193-244) | 73.0 (63.2-82.2) | 107 (94.5-127) | 197 (175-238) | 459 (372-513) | 852 (693-1010) | 1220 (1040-1410) | 732 |



## Molecular epidemiology, genetics & public health

**This is not only wrong for public health reasons,**

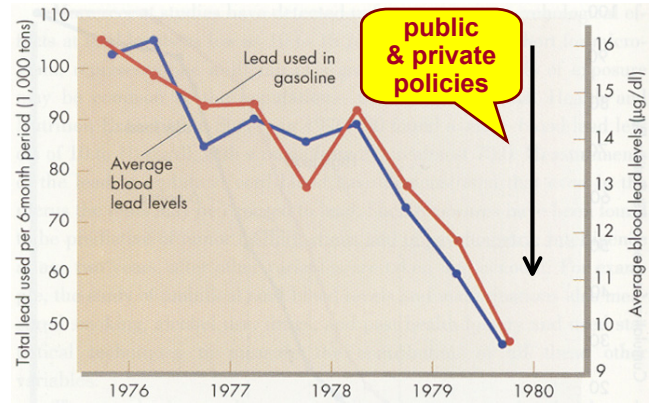
**it is also weak on clinical and biological grounds.**

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

**And only a minority of research deals with:**
**a) population impact of reducing environtl. exposures.**
**b) causes of acquired genetic alterations.**
**b1) envir. exposures as causes of acquired gene alters.**

*Gac Sanit. 2005;19(4):273-6*






**Stolley PD & Lasky T.** ***Investigating disease patterns. The science of epidemiology.*** **New York: Scientific American Library, 1995.**




http://www.who.int/reproductive-health/artforhealth/gallery.htm

www.thelancet.com Vol 368 December 16, 2006

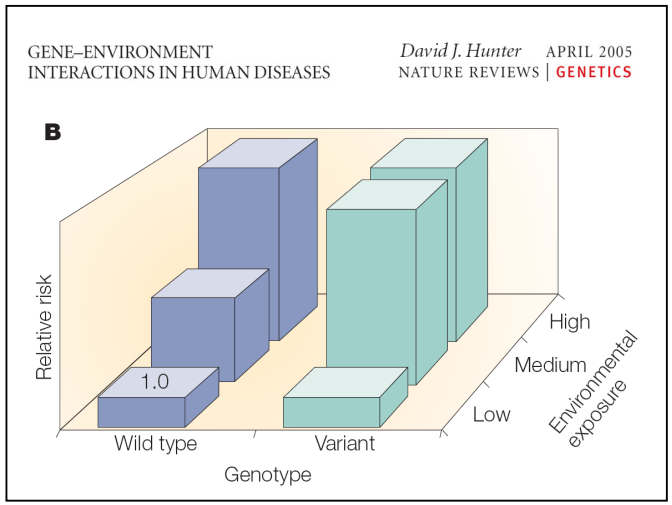
## Disease-related genetic alterations:

- Somatic or acquired
- Germinal or inhereted
  - of low penetrance
  - of high penetrance

External factors

Clinical disease








**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 supressor & 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

| Exposure | Disease | Proportion attributable to exposure |
|---|---|---|
| Tobacco smoke | Lung cancer | 90% |
| | Bladder cancer | 70% (men)<br>30% (women) |
| | Larynx cancer | 90% |
| | Coronary Heart D | 12.5% |
| | Chronic bronchitis | 80% |

Misconceptions about the use of genetic tests in populations
*Paolo Vineis, Paul Schulte, Anthony J McMichael*
THE LANCET • Vol 357 • March 3, 2001: 709-12



## B: One disease resulting from many low-penetrant genes

| Disease | Low-penetrant genes | Odds ratio |
|---|---|---|
| Lung cancer | CYP1A1 Msp I | 1.73 (Asian) |
| | | 1.04 (white) |
| | CYP1A1 exon 7 | 2.25 (Asian) |
| | | 1.30 (white) |
| | CYP2D6 | 1.26 |
| | GSTM1 | 1.34 |
| Bladder cancer | NAT-2 slow | 1.37 |
| | GSTM1 | 1.57 |
| Colon cancer | NAT-2 rapid | 1.19 |

Misconceptions about the use of genetic tests in populations
*Paolo Vineis, Paul Schulte, Anthony J McMichael*
THE LANCET • Vol 357 • March 3, 2001: 709-12



## 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 | | |
|---|---|---|---|
| | Penetrance | Frequency | Population |
| | LOW- penetrant or HIGHLY-penetrant | COMMON or RARE | GENERAL POPULATION or SOME FAMILIES |

| Las 3 situaciones reales | GENETIC TRAIT | | |
|---|---|---|---|
| | A | B | C |
| | LOW- penetrant and COMMON in the GENERAL POPULATION | HIGHLY-penetrant and COMMON in some FAMILIES | HIGHLY-penetrant and RARE in the GENERAL POPULATION |

Prevalence of carriers

Identification leads to risk reduction of

Lifetime risk of disease of carriers

Absolute Risk Reduction

**Absolute Risk Reduction (ARR)**

**NNT = 1 / ARR**

**NNS = NNT / Pr Carriers**



**NUMBER NEEDED TO SCREEN to prevent 1 case**

| | GENETIC TRAIT | | |
|---|---|---|---|
| | A | B | C |
| | LOW- penetrant and COMMON in the GENERAL POPULATION | HIGHLY-penetrant and COMMON in some FAMILIES | HIGHLY-penetrant and RARE in the GENERAL POPULATION |
| Prevalence of carriers | 13.8 per 100 | 50 per 100 | 0.16 per 100 |
| Identification leads to risk reduction of | 58 per 100 | Same as A | Same as A & B |
| Lifetime risk of disease of carriers | 14 per 1,000 | 37 per 100 | Same as B |
| Absolute Risk Reduction | 14 * 0.58 = 8 **8 per 1,000** from 14‰ to 6‰ | 37* 0.58 = 21.5 **21.5 per 100** from 37% to 15.5% | Same as B |
| NNT | 1,000 / 8 = 125 | 100 / 21.5 = 4.5 | Same as B |
| NNS | 125 / 0.138 = 906 | 4.5 / 0.5 = 9 | 4.5 / 0.0016 = 2.813 |

Vineis P et al. Lancet 2001



**NUMBER NEEDED TO SCREEN to prevent 1 case**

| | A | |
|---|---|---|
| | LOW- penetrant and COMMON in the GENERAL POPULATION | |
| Prevalence of carriers | 13.8 per 100 | Absolute Risk Reduction (ARR) |
| Identification leads to risk reduction of | 58 per 100 | 14‰ – 6‰ = 8‰ |
| Lifetime risk of disease of carriers | 14 per 1,000 | NNT = 1 / ARR |
| Absolute Risk Reduction | 14 * 0.58 = 8 **8 per 1,000** from 14‰ to 6‰ | = 1‰ / 8 = 125 |
| NNT | 1,000 / 8 = 125 | |
| NNS | 125 / 0.138 = 906 | NNS = NNT / Pr Carriers |



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

# Genetic Testing for Sale

*Paolo Vineis* 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?"

Ad: "Order your genetic test kit today"
"Preventive health profile" • $50 • 19 genes
"...You have a favorable profile
that helps fight oxidative stress..."

**U.S. Government Accountability Office (GAO)**
Nutrigenic testing • July 27, 2006
**"...That industry represents a fraudulent mutation of the genetics industry..."**



## 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 unfortunate 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

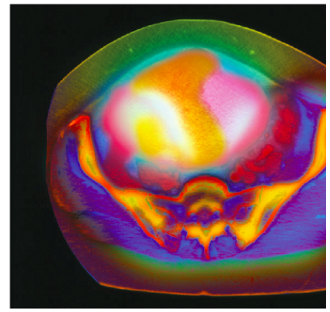


news feature

Nature, 3 June 2004

## Running before we can walk?

Two years ago, a new proteomic test was heralded as the future of cancer diagnostics. But since then, doubts about its effectiveness have begun to grow. Erika Check reports.



On target: can proteins in the blood reveal ovarian tumours (pink/yellow) before they

Seldom does a single piece of research prompt the US Congress to pass a resolution urging continued funding to drive a new diagnostic test towards the clinic. But that's what happened in 2002, when *The Lancet* published a paper[1] claiming a breakthrough in the diagnosis of ovarian cancer.

*Lancet* paper. In November 2002, Correlogic granted licences to two larger firms, Quest Diagnostics and the Laboratory Corporation of America, which are now hoping to market the test under the brand name OvaCheck.

But those plans could be thrown off track by reanalyses of Liotta and Petricoin's data by

*Bioinformatics*[2]. They had re
set that Liotta and Petricoin
online in August 2002. Sor
similarly found numerous di
protein patterns that discrimi
the cancer patients and the h
The trouble, according to So



Science, 22 October 2004

SPECIAL SECTION

GENES IN ACTION

NEWS

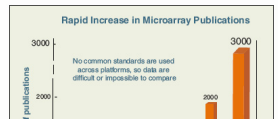
## Getting the Noise Out of Gene Arrays

**Thousands of papers have reported results obtained using gene arrays, which track the activity of multiple genes simultaneously. But are these results reproducible?**

When Margaret Cam began hunting for genes that are turned up or down in stressed-out pancreas cells a couple of years ago, she wasn't looking for a scientific breakthrough. She was shopping. As director of a support lab at the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK), she wanted to test-drive manufactured devices called microarrays or gene arrays that measure gene expression; she had her eye on three different brands. These devices are hot, as they provide panoramic views of the genes that are active in a particular cell or tissue at a particular time.

being highly up- or down-regulated.

The disharmony appears in a striking illustration in Cam's 2003 paper in *Nucleic Acids Research*. It shows a Venn diagram of overlapping circles representing the number of genes that were the most or least active on each device. From a set of 185 common genes that Cam selected, only four behaved consistently on all three platforms—"very low concordance," she said at an August forum in Washington, D.C., run by the Cambridge Healthtech Institute, based in Newton Upper Falls, Massachusetts. Using less rigorous criteria, she found about 30%




agreement—but never more than 52% between two brands. "It was nowhere near what we would expect if the probes were assaying for the same genes."

Cam's findings caused "one's jaw to drop," says Marc

he gathered on kidney tumor cells, the less significant it seemed.

But those who have persevered with gene expression arrays attribute such problems to early growing pains. They claim that experienced labs are already delivering useful clinical information—such as whether a breast cancer patient is likely to require strong chemotherapy—and that new analytical methods will make it possible to combine results from different experiments and devices. Francis Barany of Cornell University's Weill Medical College in New York City insists that arrays work well—if one digs deeply into the underlying biology.

**Imperfections**

Digging into the biology is just what Cam did after her experiments produced reams of discordant data. She and colleagues in Marvin Gershengorn's group at NIDDK wanted to pick out a set of key genes active in pancreatic tumor cells undergoing differentiation. From there, they meant to go on to examine how islet cells develop. "We were very surprised," she recalls, when they couldn't cross-validate results from studies done with Affymetrix, Agilent, and Amersham arrays. So she

Rules of evidence for cancer molecular-marker discovery and validation

*David F. Ransohoff*

NATURE REVIEWS | CANCER | APRIL 2004

Bias as a threat to the validity of cancer molecular-marker research

*David F. Ransohoff*

NATURE REVIEWS | CANCER | FEBRUARY 2005



**Journal of Translational Medicine** BioMed Central

**Abstract** Published: 31 January 2004

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 recasting 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.



**clinical epidemiology ↔ proteomics**

ELSEVIER

Journal of Clinical Epidemiology 56 (2003) 815–819

Journal of Clinical Epidemiology

Semiology, proteomics, and the early detection of symptomatic cancer

Miquel Porta[a,b,c,*], Esteve Fernandez[d,e], Joan Alguacil[a,f]

[a]*Institut Municipal d'Investigació Mèdica, Carrer del Dr. Aiguader 80, Barcelona E-08003, Spain*
[b]*Universitat Autònoma de Barcelona, Barcelona, Spain*
[c]*School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA*
[d]*Institut Català d'Oncologia, L'Hospitalet de Llobregat, Barcelona E-08907, Spain*
[e]*Universitat de Barcelona, Barcelona, Spain*
[f]*Division of Cancer Epidemiology & Genetics, National Cancer Institute, Bethesda, MD 20892, USA*

the new wave of genomic and proteomic analyses of early-stage cancers might provide new insights into changes that occur in early phases of tumorigenesis; it is already offering new candidate biomarkers for early-stage disease [37–39].



**clinical epidemiology ↔ proteomics**

*Clin Transl Oncol. 2005;7(5):195-204*

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

Miquel Porta[a,b], Xavier Fabregat[b,c], Núria Malats[a], Luisa Guarner[d], Alfredo Carrato[c], Ana de Miguel[c],

There was a clear trend towards more localized tumours with increasing numbers of cholestatic signs ($p<0.001$).

*J Epidemiol Community Health* 2002;**56**:734–738



**Attention to selection biases !**

**DEBATE**

## 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 Carrato, M Soler, L Ruiz, V Barberà, D Ayude, F X Real

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

**Cancer Epidemiology, Biomarkers & Prevention**
*Vol. 9, 1223–1232, November 2000*

## K-*ras* and p53 in Pancreatic Cancer: Association with Medical History, Histopathology, and Environmental Exposures in a Population-based Study[1]



Joan Mitchell
Ici



*Ann Epidemiol 2002;12:7–14.*

ELSEVIER

**Attention to selection biases !**

## Generalizing Molecular Results Arising from Incomplete Biological Samples:

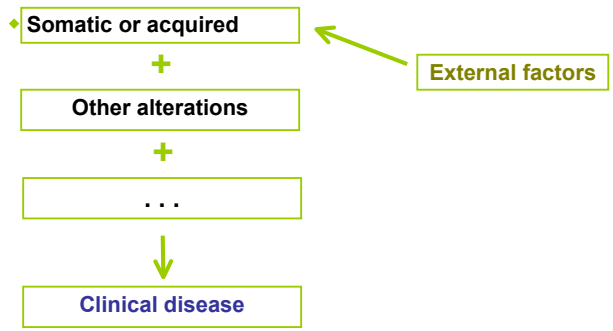
### Expected Bias and Unexpected Findings

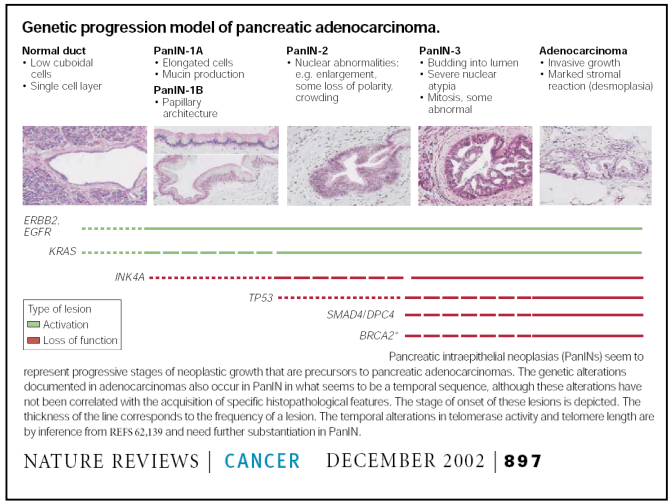
MIQUEL PORTA, MD, MPH, PHD, NÚRIA MALATS, MD, PHD, JOSEP M. COROMINAS, JULI RIFÀ, MD, PHD, JOSEP L. PIÑOL, MD, PHD, AND FRANCISCO X. REAL, MD, PHD, FOR THE PANKRAS I PROJECT INVESTIGATORS*

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



## Disease-related genetic alterations:





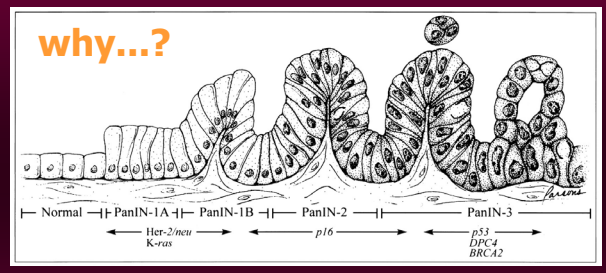



***Although a genetic profile for pancreatic cancer is emerging, many biological aspects of this disease are poorly understood. Indeed, fundamental questions regarding progenitor cell lineages, host stromal milieu, and the role of specific genetic alterations in tumor progression remain unresolved.***

**Bardeesy N et al.**
**The genetics of pancreatic adenocarcinoma: a roadmap for a mouse model**
**SEMINARS IN CANCER BIOLOGY 2001**

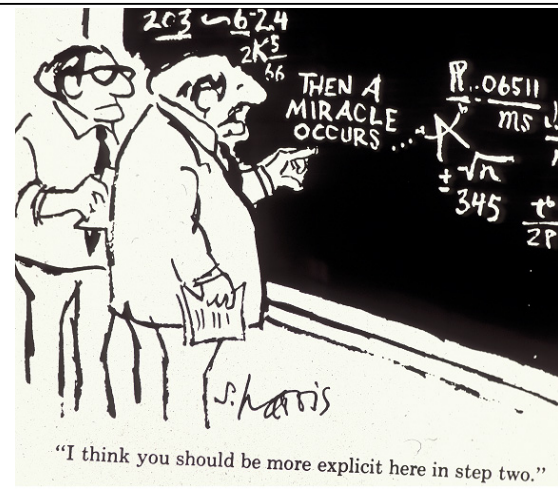
**+ Bardeesy & DePinho. *Nature Reviews Cancer* 2002.**



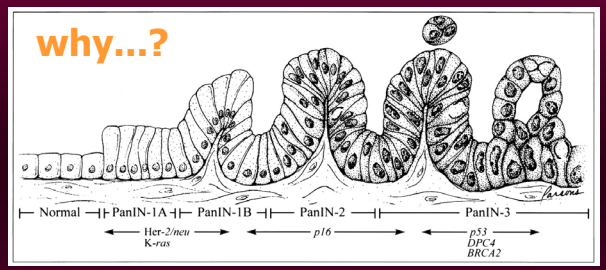



**Hruban RH, Goggins M, Parsons J, Kern SE.**
**Progression model for pancreatic cancer.**
**Clin Cancer Res 2000; 6: 2969-72.**





## why...?




**Hruban RH, Goggins M, Parsons J, Kern SE. Progression model for pancreatic cancer. Clin Cancer Res 2000; 6: 2969-72.**



## 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?**



MOLECULAR CARCINOGENESIS 36:45–52 (2003)

WORKING HYPOTHESIS

### Exploring Environmental Causes of Altered *ras* Effects: Fragmentation Plus Integration?

**Miquel Porta,[1,2]* Daniel Ayude,[1,2] Juan Alguacil,[1,3] and Manuel Jariod[1]**

[1]*Institut Municipal d'Investigació Médica, Barcelona, Spain*
[2]*Universitat Autònoma de Barcelona, Catalonia, Spain*
[3]*Occupational Epidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, Maryland*

Human cancer, carcinogenic exposures and mutation spectra

Paolo Vineis [a,*], Nùria Malats [b], Miquel Porta [b], Francisco X. Real [c]

Causal Thinking, Biomarkers, and Mechanisms of Carcinogenesis*

*Paolo Vineis[1] and Miquel Porta[2]*
[1]UNIT OF CANCER EPIDEMIOLOGY, 10126 TORINO, ITALY AND [2]INSTITUT MUNICIPAL D'INVESTIGACIÓ MÈDICA, UNIVERSITAT AUTÒNOMA DE BARCELONA, BARCELONA, SPAIN E-08003



Research | Article

### Organochlorine Exposure and Colorectal Cancer Risk

*Mike Howsam,[1] Joan O. Grimalt,[2] Elisabet Guinó,[3] Matilde Navarro,[3] Juan Martí-Ragué,[4] Miguel A. Peinado,[5] Gabriel Capellá,[3] and Víctor Moreno[3] for the Bellvitge Colorectal Cancer Group**

Organochlorine compounds have been linked to increased risk of several cancers. Despite reductions in their use and fugitive release, they remain one of the most important groups of persistent pollutants to which humans are exposed, primarily through dietary intake. We designed a case–control study to assess the risk of colorectal cancer with exposure to these chemicals, and their potential interactions with genetic alterations in the tumors. A subsample of cases ($n$ = 132) and hospital controls ($n$ = 76) was selected from a larger case–control study in Barcelona, Catalonia, Spain. We measured concentrations in serum of several organochlorines by gas chromatography. We assessed point mutations in K-*ras* and *p53* genes in tissue samples by polymerase chain reaction/single-strand conformation polymorphism and assessed expression of p53 protein by immunohistochemical methods. An elevated risk of colorectal cancer was associated with higher serum concentrations of mono-*ortho* polychlorinated biphenyl (PCB) congeners 28 and 118. The odds ratio for these mono-*ortho* PCBs for middle and higher tertile were, respectively, 1.82 [95% confidence interval (CI), 0.90–3.70] and 2.94 (95% CI, 1.39–6.20). α-Hexachlorocyclohexane, hexachlorobenzene, and *p,p'*-DDE (4,4'-dichlorodiphenyltrichloroethene) showed nonsignificant increases in risk. Risk associated with mono-*ortho* PCBs was slightly higher for tumors with mutations in the *p53* gene but was not modified by mutations in K-*ras*. Mono-*ortho* PCBs were further associated with transversion-type mutations in both genes. These results generate the hypothesis that exposure to mono-*ortho* PCBs contributes to human colorectal cancer development. The trend and magnitude of the association, as well as the observation of a molecular fingerprint in tumors, raise the possibility that this finding may be causal.
*Key words:* case–control study, colorectal cancer, K-*ras* mutations, organochlorines, *p53* mutations, PCBs. *Environ Health Perspect* 112:1460–1466 (2004). doi:10.1289/ehp.7143 available via *http://dx.doi.org/* [Online 15 July 2004]

Carcinogenesis

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



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

Miquel Porta, Núria Malats, Luisa Guarner, Alfredo Carrato, Juli Rifà, Antonio Salas, Josep M Corominas, Montserrat Andreu, Franscisco 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.**

(*J Epidemiol Community Health* 1999;**53**:702–709)



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



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

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**Mocha and mutations**

*15 December 1999*

Following recent conflicting but intriguing results thrown up by rodent and cell culture research, about the effects of caffeine and coffee, Miquel Porta of the Universitat Autonoma de Barcelona, Spain, and colleagues, carried out this new work in five Spanish hospitals. They showed for the first time that, in people suffering from pancreatic cancer, those who also had mutations in K-*ras* drank around 14 and a half cups of coffee a week-significantly more coffee than those without K-*ras* mutations, who tended to consume nearer 9 cups. Nine of the 121 patients studied drank more than 21 cups of coffee a week, and all of them had mutated tumours.



**Table 2**

*K-ras mutations and regular coffee consumption among cases of cancer of the pancreas*

| Regular coffee drinker | Yes | No | Total |
|---|---|---|---|
| K-ras mutated | 73 | 10 | 83 |
| K-ras wild type | 16 | 8 | 24 |
| Total | 89 | 18 | 107 |

*Source:* Porta et al. (1999).

Paul R. Rosenbaum
**The Case-Only Odds Ratio as a Causal Parameter**
BIOMETRICS 60, 233–240
March 2004

**"In a case-only design (...) the odds ratio in this table is taken as a measure of gene-environment interaction"**



*European Journal of Epidemiology* **18**: 289–298, 2003.

**Coffee drinking: The rationale for treating it as a potential effect modifier of carcinogenic exposures**

M. Porta[1,2], J. Vioque[3], D. Ayude[1,2], J. Alguacil[1,4], M. Jariod[1]

Caffeine can profoundly alter cell cycle checkpoint function and several mechanisms of DNA repair, as well as carcinogen metabolism. The impact of caffeine on cell cycle checkpoint function occurs in spite of it being nonmutagenic in traditional mutagenesis assays.

The study of interactions between caffeine-containing beverages and environmental agents in well defined groups of healthy and diseased people could yield new insights into checkpoint signal transduction and other mechanisms of carcinogenesis.

PORTO CANCER MEETING - "Cancer etiology: bridging worlds"
IPATIMUP, Universidade do Porto (Portugal), 20-21 abril 2007
Miquel Porta -- página 44

*European Journal of Epidemiology* **18**: 289–298, 2003.

**Coffee drinking: The rationale for treating it as a potential effect modifier of carcinogenic exposures**

The impact of caffeine on cell cycle checkpoint function occurs in spite of it being nonmutagenic in traditional mutagenesis assays. In this respect caffeine might resemble a number of chemicals found in the environment that do not show mutagenic properties in a variety of assays, yet affect cell surveillance, and may even have the ability to induce tumours in rodents [23].

It has been hypothesised that a nongenotoxic environmental carcinogen may function by ablating some aspect of cell cycle checkpoint function, perhaps leading to genetic instability or heritable alterations of the genome. The study of such environmental chemical agents may give insight into checkpoint signal transduction and mechanisms of carcinogenesis [23].

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

MOLECULAR CARCINOGENESIS 36:45–52 (2003)

WORKING HYPOTHESIS

# Exploring Environmental Causes of Altered *ras* Effects: Fragmentation Plus Integration?

**Miquel Porta,[1,2]* Daniel Ayude,[1,2] Juan Alguacil,[1,3] and Manuel Jariod[1]**

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Organochlorine compounds, organic solvents, and coffee compounds may play an indirect role in causing Ki-*ras* mutations, rather than as direct inducers of the mutations.

Although for some organochlorine compounds the induction of point mutations in *ras* oncogenes cannot be excluded, it seems more likely that the effects of these compounds are mediated through nongenomic or indirectly genotoxic mechanisms of action. Organic solvents also may act via enzymatic induction of *ras* mutagens or by providing a proliferation advantage to *ras*-mutated cell clones. In exocrine pancreatic cancer, caffeine, other coffee compounds, or other factors with which coffee drinking is associated could modulate Ki-*ras* activation by interfering with DNA repair, cell-cycle checkpoints, and apoptosis.



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Might connections exist between the environmental exposures that we found to be associated with Ki-*ras* mutations and some of the agents and processes controlling *ras* status and function? Our previous papers [22,23,29–31] sketch several mechanistic scenarios that could help address this question, offering ideas about possible factors that might play direct and indirect roles in Ki-*ras* activation, in preventing repair of such mutations, or in providing a growth advantage to *ras*-mutated cells. A summary of such mechanistic scenarios follows.



THE LANCET

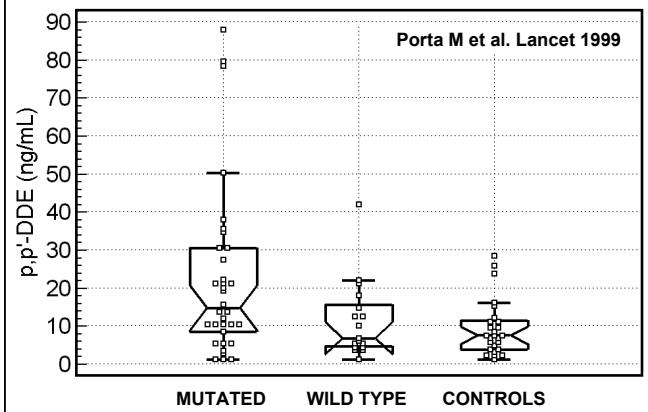
EARLY REPORT

2125 **Serum concentrations of organochlorine compounds and K-*ras* mutations in exocrine pancreatic cancer**
M Porta and others, for the PANKRAS II Study Group

Volume 354, Number 9196 · Founded 1823 · Published weekly · Saturday 18/25 December 1999

Several organochlorine compounds can act as carcinogens and tumour promoters.[3–8] Some modulate the expression of oncogenes, including *ras* genes.[9,10] DDT and some PBCs have endocrine effects.[1,2,11,12] Although presumably weak, such effects may be enhanced by environmental biodegradation, the long half-lives of the compounds (about 10 years for DDE, 30 years or more for some PCBs), and their concentrations in target tissues (100-fold to 350-fold higher in adipose tissue than in blood).[1,5,6]

PORTO CANCER MEETING - "Cancer etiology: bridging worlds"
IPATIMUP, Universidade do Porto (Portugal), 20-21 abril 2007
Miquel Porta -- página 46




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

**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.**



THE LANCET

51 cases of EPC (+ 26 controls)
adjust. by coffee, tobacco, alcohol

**EARLY REPORT**

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**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.

| | OR | P-value (OR 95% CI) |
|---|---|---|
| **p,p'-DDT** | | |
| ≤224 (ng/g) | **1.0** | **0.009** |
| 225 – 614 | **39.8** | (3.7-443.1) |
| >614 | **3.1** | (0.8-12.3) |
| **Coffee** | | |
| Non reg. drinkers | **1.0** | **0.111*** |
| 1-7 cups/week | **3.8** | (0.7-20.0) |
| 8-14 cups/week | **2.4** | (0.4-13.9) |
| ≥15 cups/week | **4.5** | (0.9-22.7) |

| | | |
|---|---|---|
| ≤190 | **1.0** | **<0.001** |
| >190 | **10.5** | (2.9-38.2) |

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

PORTO CANCER MEETING - "Cancer etiology: bridging worlds"
IPATIMUP, Universidade do Porto (Portugal), 20-21 abril 2007
Miquel Porta -- página 48

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| ≥15 cups/week | **4.5** | (0.9-22.7) |

| Regular coffee drinkers | | |
|---|---|---|
| No | **1.0** | **0.038** |
| Yes | **4.1** | (1.1-15.5) |

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

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 effect of PCB 153 and coffee
on the probability of a mutated (vs. wild-type) tumour.

| | OR | P-value (OR 95% CI) |
|---|---|---|
| **PCB 153** | | |
| ≤187 (ng/g) | **1.0** | **0.003*** |
| 188 – 313 | **3.9** | (1.0-15.0) |
| >313 | **11.4** | (2.3-57.4) |
| **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) |

| | | |
|---|---|---|
| ≤305 | **1.0** | **0.016** |
| >305 | **5.5** | (1.4-22.2) |

ORs further adjusted by age, sex and cholestatic 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.

| | OR | P-value (OR 95% CI) |
|---|---|---|
| **p,p'-DDT** | | |
| ≤224 (ng/g) | **1.0** | **0.032** |
| 225 – 614 | **23.4** | (2.0-267.3) |
| >614 | **1.2** | (0.2-6.3) |
| **PCB 153** | | |
| ≤187 (ng/g) | **1.0** | **0.029*** |
| 188 – 313 | **4.8** | (0.8-28.4) |
| >313 | **9.3** | (1.3-66.6) |
| **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.



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| 8-14 cups/week | **8.4** | (1.5-45.6) |
| ≥15 cups/week | **8.0** | (1.5-41.6) |

| Regular coffee drinkers | | |
|---|---|---|
| No | **1.0** | **0.006** |
| Yes | **6.6** | (1.7-25.3) |

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



## Conclusions / 1

**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.**



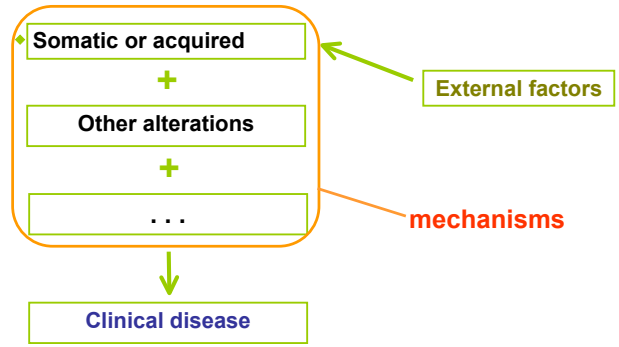
## So what?

**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:



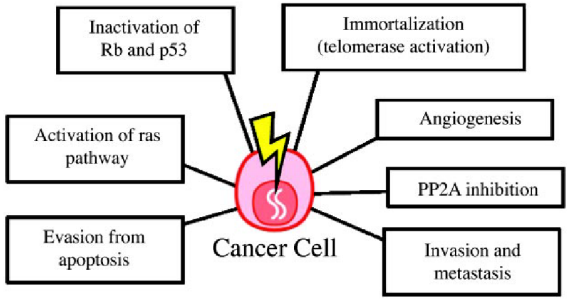



Fig. 1. Malignant growth requires the inactivation of cellular tumor suppressor genes such as Rb, p53, and possibly others, activation of growth stimulatory pathways, such as ras, phosphorylation changes of several cellular proteins such as those obtained by inactivating phosphatase 2A, evasion from apoptosis, immortalization, angiogenesis, and invasion and metastasis. Moreover, interactions among the malignant cells with the tissue stroma and the immune system will influence tumor growth.

[CANCER RESEARCH 64, 5518–5524, August 1, 2004]



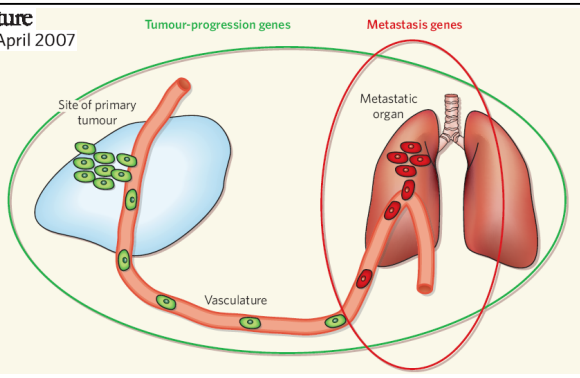



**Figure 1 | Roles of different genes in cancer.** Gupta *et al.*[4] identify genes that are required for the growth of primary tumours, for intravasation and extravasation of tumour cells to the specific metastatic target organ (as exemplified here by the lung) and for metastatic outgrowth. These genes are required for the growth of the primary tumour and the many stages of metastasis, and can be classified as 'tumour-progression genes'. By contrast, 'metastasis genes' contribute exclusively to metastatic outgrowth.



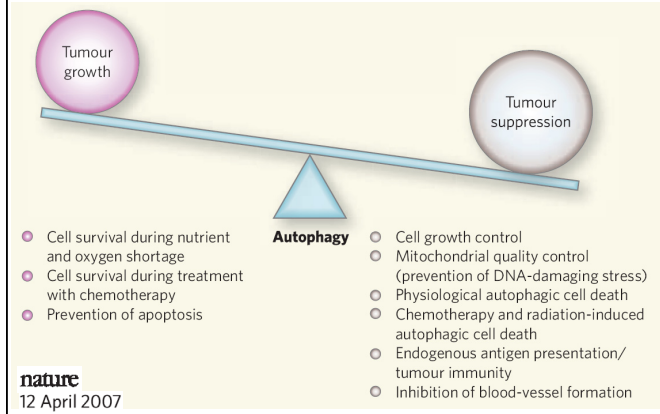
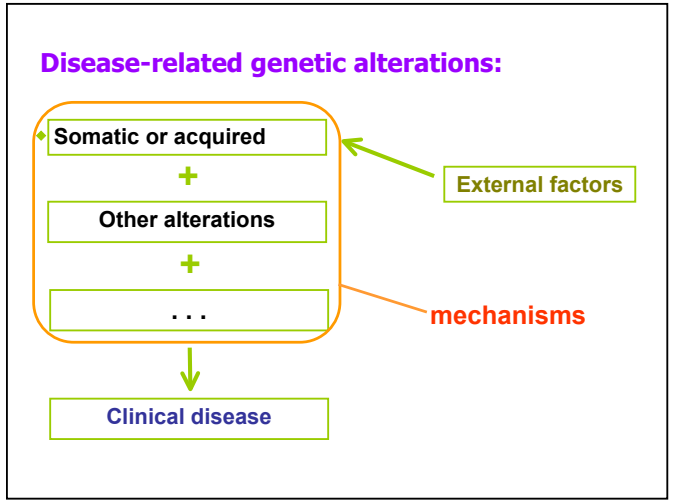



**Figure 3 | Conflicting effects of autophagy on cancer.** Experimental evidence supports a role for autophagy in both cancer development and suppression. What tips the balance is not clear.





**Disease-related genetic alterations:**

The renowned "black box"

External factors

mechanisms

Clinical disease



**Disease-related genetic alterations:**

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

External factors

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

mechanisms

Clinical disease



**Attention to mechanisms**
**Attention to interactions**
**Attention to indirect effects (e.g., epigenetic effects)**

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

**NOT negligible:**
**↑↑↑ number of individuals exposed to environmental chemical agents**

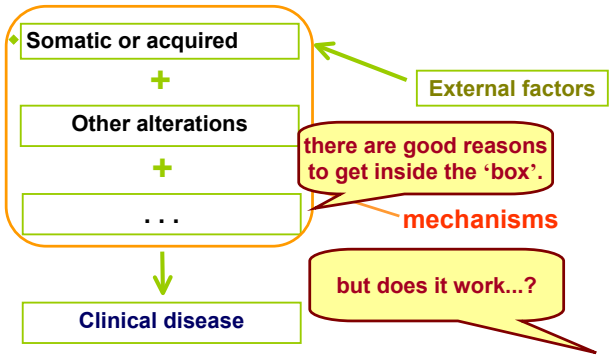


www.cdc.gov/exposurereport

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

| | Geometric mean (95% conf. Interval) | Selected percentiles (95% confidence interval) | | | | | | Sample size |
|---|---|---|---|---|---|---|---|---|
| | | 10th | 25th | 50th | 75th | 90th | 95th | |
| Total, age 12 and older | 260 (234-289) | 74.2 (66.1-84.2) | 114 (99.8-129) | 226 (191-267) | 538 (485-609) | 1120 (991-1290) | 1780 (1520-2230) | 1964 |
| Age group | | | | | | | | |
| 12-19 years | 118 (101-137) | 45.9 (34.9-56.6) | 69.8 (59.2-80.4) | 108 (90.6-132) | 185 (141-233) | 343 (255-479) | 528 (364-644) | 686 |
| 20 years and older | 297 (267-330) | 86.0 (75.2-96.7) | 130 (115-150) | 269 (229-303) | 626 (538-697) | 1250 (1100-1420) | 1990 (1570-2510) | 1278 |
| Gender | | | | | | | | |
| Males | 249 (221-281) | 77.6 (68.6-88.2) | 119 (101-133) | 222 (182-266) | 489 (383-570) | 985 (756-1130) | 1350 (1190-1610) | 937 |
| Females | 270 (241-302) | 68.9 (55.1-82.5) | 112 (96.0-129) | 228 (191-286) | 604 (516-697) | 1320 (1100-1600) | 2150 (1650-2750) | 1027 |
| Race/ethnicity | | | | | | | | |
| Mexican Americans | 674 (572-795) | 154 (133-214) | 300 (252-370) | 623 (505-750) | 1350 (1090-1660) | 3090 (2100-4610) | 4940 (3280-7810) | 657 |
| Non-Hispanic blacks | 295 (253-344) | 62.2 (56.9-80.5) | 113 (98.3-128) | 203 (164-253) | 452 (392-571) | 1340 (974-1910) | 2160 (1470-4010) | 416 |
| Non-Hispanic whites | 217 (193-244) | 73.0 (63.2-82.2) | 107 (94.5-127) | 197 (175-238) | 459 (372-513) | 852 (693-1010) | 1220 (1040-1410) | 732 |

## Disease-related genetic alterations:






The New York Times **Health**
February 3, 2004

# New Cancer Test Stirs Hope and Concern

By ANDREW POLLACK

Jill Doimer's mother died in 2002 from ovarian cancer, detected too late to be effectively treated.

So Ms. Doimer is eagerly awaiting the introduction of a new test that holds the promise of detecting early-stage ovarian cancer far more accurately than any test available now, using only blood from a finger prick.

Not only does she plan to be tested, but an advocacy group she helped found, Ovarian Awareness of Kentucky, also intends to spread the word to women and doctors.

"If it's going to happen to me or anyone I know, I want it to be caught at an early stage," said Ms. Doimer, who lives in Louisville.

The new test, expected to be available in the next few months, could have a big effect on public health if it works as advertised. That is because when ovarian cancer is caught early, when it is treatable by surgery, more than 90 percent of women live five years or longer. But right now, about three-quarters of cases are detected after the cancer has advanced, and then only 25 percent of women survive five years.

The test is also the first to use a new technology that some believers say could revolutionize diagnostics. It looks not for a single telltale protein — like the prostate-specific antigen, or P.S.A., used to diagnose prostate cancer — but rather for a complex fingerprint formed by all the proteins in the blood. Similar tests are being developed for prostate, pancreatic, breast and other cancers. The technique may work for other diseases as well.

"I've been in cancer research for 40 years and I think it's the most important breakthrough in those years," said Dr.

*Continued on Page 6*



The New York Times **Health**
February 3, 2004

## New Cancer Test Stirs Hope and Concern

By ANDREW POLLACK

**Dr. Emmanuel F. Petricoin III, an agency scientist who helped develop OvaCheck, said the criticisms of it were based "in some instances on not understanding the entirety of the science."**

**"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.**



The New York Times **Health**
February 3, 2004

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**The test is also the first to use a new technology that some believers say could revolutionize diagnostics. It looks not for a single telltale protein -like the prostate-specific antigen, or P.S.A., used to diagnose prostate cancer- but rather for a complex fingerprint formed by all the proteins in the blood. Similar tests are being developed for prostate, pancreatic, breast and other cancers.**

**Some experts, however, say that the technique, while promising, is still unproved. They say the ovarian test in particular has not been adequately validated and is being put on the market prematurely through a route that does not require approval by the Food and Drug Administration. If the test is not accurate, they say, it could result in unnecessary surgery for biopsies or ovary removal for many women.**

The New York Times **Health**
February 3, 2004

**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.**

**Back to the black-box?**

**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.**



The New York Times **Health**
February 3, 2004

**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** [illegible]**ith Dr. Lance A. Liotta, who helped**

**Back to the black-box?**

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.



The New York Times **Health**
February 3, 2004

## New Cancer Test Stirs Hope and Concern

By ANDREW POLLACK

**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.**



## Disease-related genetic alterations:

- **Somatic or acquired**

**External factors**

- **Often studies are restricted to inhereted alterations in low penetrance genes, and researchers overlook how certain environmental processes interact biologically with the genetic material and cause mutations.**

Cancer Epidemiol Biomarkers Prev 2004;13(12). December 2004

*Editorial*

**An excellent piece, except that...**

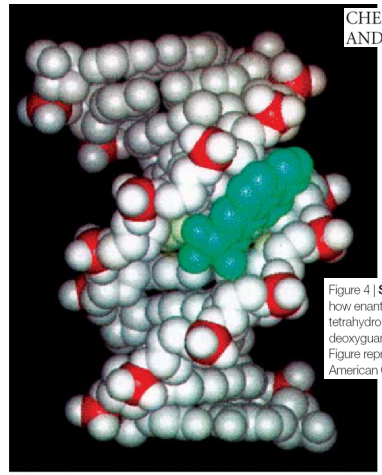
## Genetic Variation and Cancer: Improving the Environment for Publication of Association Studies

Timothy R. Rebbeck,[1] María Elena Martínez,[2] Thomas A. Sellers,[3] Peter G. Shields,[4] Christopher P. Wild,[5] and John D. Potter[6]

[1]Abramson Cancer Center, Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania; [2]Arizona Cancer Center, University of Arizona, Tucson, Arizona; [3]Moffitt Cancer Center and University of South Florida, Tampa, Florida; [4]Lombardi Comprehensive Cancer Center, Georgetown University School of Medicine, Washington, District of Columbia; [5]Molecular Epidemiology Unit, University of Leeds, Leeds, United Kingdom; and [6]Fred Hutchinson Cancer Research Center, Seattle, Washington

- **Often studies are restricted to inhereted alterations in low penetrance genes, and researchers overlook how certain environmental processes interact biologically with the genetic material and cause mutations.**





CHEMICAL-INDUCED DNA DAMAGE AND HUMAN CANCER RISK

*Miriam C. Poirier*

NATURE REVIEWS | CANCER
AUGUST 2004

Figure 4 | **Structure of BPdG.** This space-filling model shows how enantiomeric 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-benzo[*a*]pyrene (BPDE; green) binds to deoxyguanine and fits into the minor groove of the DNA helix. Figure reproduced with permission from REF. 30 © (1997) American Chemical Society.



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

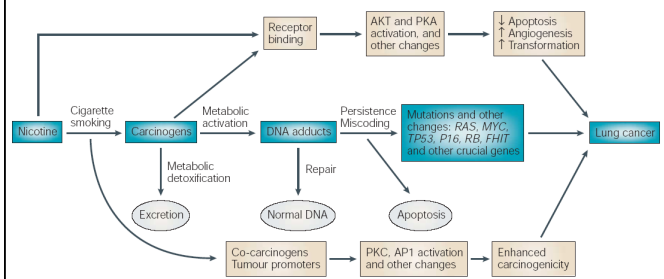
1) **They are real physico-chemical interactions with DNA.**

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...)**



2) **The environ. factor CAUSES ➡ the genetic alteration.**




TOBACCO CARCINOGENS, THEIR BIOMARKERS AND TOBACCO-INDUCED CANCER

NATURE REVIEWS | CANCER
OCTOBER 2003
*Stephen S. Hecht*

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

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

...?



**Molecular epidemiology, genetics & public health**

**This is not only wrong for public**

...?

**it is also weak on clinical and biological grounds.**

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

**And only a minority of research deals with:**
**a) population impact of reducing environtl. exposures.**
**b) causes of acquired genetic alterations.**
**c) how envir. exposures cause acquired gene alterats.**

*Gac Sanit. 2005;19(4):273-6*



**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*
- Genetic biomarkers and occupational epidemiology –recollections, reflections and reconsiderations. SJWEH 2004; 30: 1-3.
- Promise of molecular epidemiology –epidemiologic reasoning, biological rationale and risk assessment. SJWEH 1999; 25: 498-504.

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

1. The variant is inhereted and thus non-modifiable.
2. The variant has no clinical impact if there is no exposure.
3. The socialisation of studies atenuates 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.



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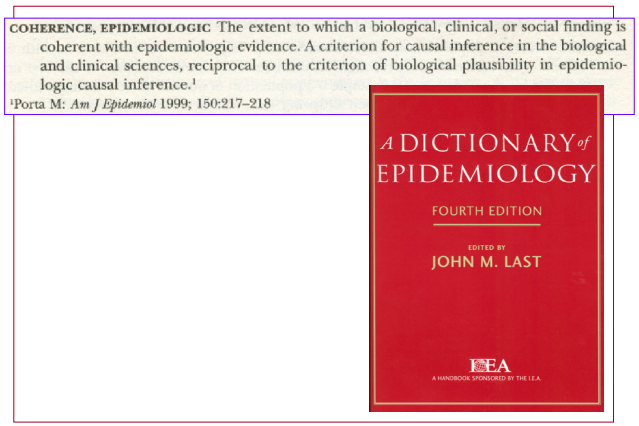
**9 Limitations of studies on inherited genetic variants of low penetrance:**

**9.** Low biologic and epidemiologic coherence 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...



**Other essential problems (methodological, epistemological & ontological):**

- **Underestimation of causal complexity.**

**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...

*European Journal of Epidemiology* **19**: 733–735, 2004.


**Epidemiologic methods: Beyond clinical medicine, beyond epidemiology**

Francisco Bolúmar[1] & Miquel Porta[2]
[1]*Universitat Miguel Hernández, Alacant, Spain;* [2] *Institut Municipal d'Investigació Mèdica and Universitat Autónoma de Barcelona, Spain*

**It is unimportant that the language sounds too ecclesiastic to us.**

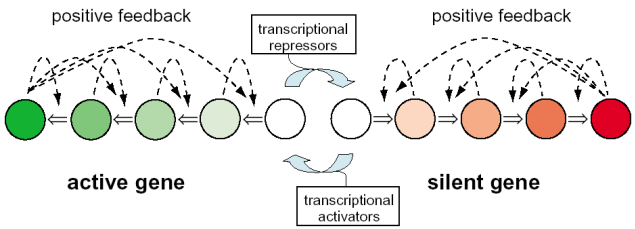
**We simply find such propositions at odds with much of the contemporary scientific world: wide open, transdisciplinary –much more creative, relevant, efficient, and interesting because of the porousness, flexibility and adaptability of the disciplines than because of the putative highers mission of 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 loosing 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?'**






Rudolf Jaenisch[1] & Adrian Bird[2]

**Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals**

**nature genetics supplement • volume 33 • march 2003**

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

Histone (H3) modifications include lysine (K) acetylation (Ac) and lysine methylation (Me). Lysines at other positions are also modified. The HP1 protein recognizes MeK9 and, as this protein also binds the histone methyltransferase (HMT), heterochromatin can spread. Histone deacetylases (HDAC) deacetylate lysine residues as a prerequisite for their subsequent methylation. DNA methyltransferases (DNMT) participate in multiprotein complexes that contain HDACs and HMTs, and methyl-C binding proteins (MBD) can be loaded onto methylated DNA through their interactions with both HDACs and HMTs. Much of the evidence comes from studies of constitutive heterochromatin, but recent studies indicate similar interactions of genes silenced *de novo* in cancer cells.

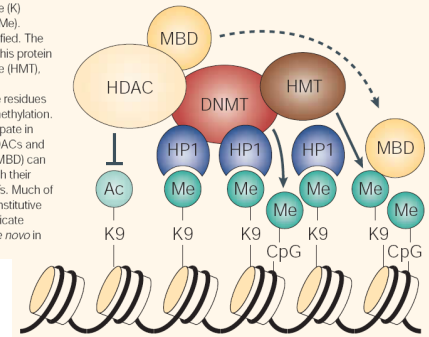



Figure 2 | **Co-operative and self-reinforcing organization of the chromatin and DNA-modifying machinery responsible for gene silencing in normal and malignant cells.**

NATURE REVIEWS | CANCER | FEBRUARY 2004



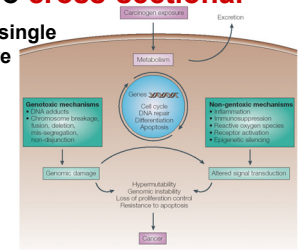
**Other essential problems (methodological, epistemological & ontological):**

- **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...**

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nature
Vol 441 | Issue no. 7092 | 25 May 2006

## Coping with complexity

Two philosophers of science recently surveyed 500 geneticists to ask their opinion on whether 14 different sets of genetic information constituted a gene, or more than one gene. Fortunately, the bulk of the respondents felt able to answer the questions definitively. Less fortunately, their answers were inconsistent, with the sample often quite evenly split on the question of how many genes were actually present.

## Policing ourselves

**Investigación biomédica y sociedad: ambivalencias y contradicciones**

Miquel Porta *Med Clin (Barc).* 2007;128



**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**




*Figure 1: Same sky, same women*
*Elisabetta Farina, 2006, acrylic on canvas.*

World Health Organization

**www.elisabettafarina.com**

http://www.who.int/reproductive-health/artforhealth/gallery.htm

www.thelancet.com Vol 368 December 16, 2006

PORTO CANCER MEETING - "Cancer etiology: bridging worlds"
IPATIMUP, Universidade do Porto (Portugal), 20-21 abril 2007
Miquel Porta -- página 72

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