

## **Commentary**

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## **Gene splicing and pie slicing: causal models for the omics age.**

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## Summary

The explosion of research on genetic factors in disease presents a tremendous opportunity to epidemiologists to improve our ability to identify preventable causes of ill health. Epidemiologists have enthusiastically embraced the new tools of genomics and proteomics, and pressed them into service to investigate gene-environment interactions (GEI). We argue that neither the full import nor limitations of GEI studies can be appreciated without clarifying underlying theoretical models of interaction, etiologic fraction, and indeed the fundamental concept of causality. In this paper we explore different models for the explanation of cause in the epidemiology of disease arising out of genes, environments and the interplay between environments and genes. We begin from Rothman's "pie" model of necessary and sufficient causes, and then discuss several newer methods which provide additional insights into multifactorial causal processes. These include directed acyclic graphs (DAG) and structural equation models. Caution is urged in the application of two essential and closely related concepts found in many GEI studies: interaction (effect modification) and the etiologic or attributable fraction. We review these concepts and present several important limitations. These are:

1. Interaction is a fundamental characteristic of any causal process involving a series of probabilistic steps, and not a second-order phenomenon identified after first accounting for "main effects".

2. Standard methods of assessing interaction do not adequately consider the life course, and the temporal dynamics through which an individual's sufficient cause is completed. Recent genetic research suggests that different individuals are at different stages of development along the path to disease, but this is not usually measurable. We illustrate this with a brief review of early susceptibility in children, which demonstrates that acquired susceptibility, even *in utero*, can be an important source of variation in susceptibility.

3. Whenever discussing a causal mechanism, one must be careful to distinguish individual- and population-level models. Most epidemiologic discussions of causality fail to make this distinction.

4. At the population level, there is additional uncertainty in quantifying interaction and assigning etiologic fractions to different necessary causes because of ignorance about the components of the sufficient cause.

## **Introduction**

It is a matter of considerable importance for those charged with setting health care priorities to know how large a burden of disease should be attributed to a particular preventable cause. The case of the occupational burden of cancer, for example, has been the subject of a number of recent studies [1, 2]. Unfortunately, there are substantial uncertainties in nearly all the data that are used in making these estimates. The key pieces of data are: 1) a list of all of the known or suspected carcinogens in the workplace; 2) the prevalences of exposures to these agents; and 3) information on the magnitudes of the risks of different types of tumors from the various exposures (exposure response curves). Major gaps exist in knowledge of all three of these, and so considerable humility is called for when assigning a figure to the occupational cancer burden. This paper however, focuses on additional uncertainties of a more fundamental nature: how to factor into such estimates the possibility (or indeed strong likelihood) that multiple cancer risk factors interact in ways that we do not understand, preventing a simple summing of the carcinogenic contributions of different causes. The challenge is not limited to cancer studies: the problem of identifying and quantifying multiple component causes of disease is one of the most basic limitations in modern epidemiology.

## **1. Concepts of ‘causality’ in medicine**

### **1.1. From Koch to Rothman**

In a rather simplified way, causation involves the relationship between at least two entities, an agent and a disease. We can describe at least two distinct eras in the history of medical causality in the last two centuries. The first era corresponds to the ‘microbiological’ revolution, i.e. the triumph of a linear monocausal (Aristotelian) concept of cause. After the work of Pasteur and Koch, the agent of a disease came to be conceived of as a single necessary cause (e.g. Mycobacterium for tuberculosis). The concept of necessary cause means that the disease does not develop in the absence of exposure to the agent. Such a view implies: a) that the cause is, at least potentially, definable unequivocally and is easily identifiable; b) that the disease can be also defined unequivocally, i.e. it is not a complex and variable constellation of symptoms. Clearly there are some conditions in which the relationship between a (necessary) cause and the corresponding disease is indeed evident: for example, smallpox is a clear cut disease entity, easy to define and diagnose; it is due to a single necessary virus (no smallpox develops in the absence of the specific virus); and clear proof of the causal link has come from the disappearance of smallpox after large scale vaccination.

Cases such as smallpox are however in the minority. More frequently, in the “Pasteur-Koch” paradigm we find a clearly defined agent (usually a bacterium, parasite or virus) which is used as

the “unifying element” of a *constellation* of symptoms, i.e. the disease itself is largely defined and recognized on the basis of the agent. The popularity of the “Pasteur-Koch” approach to causality has not decreased however, and the concept of a necessary cause of disease is still discussed as a universal paradigm in medicine.

The second era in the history of causation in medicine arises out of the study of chronic diseases like cancer or cardiovascular disease. In these cases the concept of a “necessary” condition is rarely, if ever, meaningful. No “necessary” cause of cancer is known (with the possible exception of human papilloma virus and cervical cancer); rather, in such cases, the idea of a “causal web” has been introduced and widely applied [3]. The causal web reflects the fact that a concurrence of different “exposures” or conditions is required to induce disease, none of which is in itself necessary. For example, lung cancer can be induced by a causal web including tobacco smoking and individual predisposition from the CYP1A1 and other high-risk genotypes [4]. Another causal web may be represented by asbestos exposure and low consumption of raw fruits and vegetables in the occurrence of mesothelioma. The idea of the web implies then, that while the disease is usually well defined from a clinical point of view (e.g. lung cancer or mesothelioma), the etiologic perspective is more complex: not all lung cancer cases can be linked to the same exposures, but may instead share partially overlapping constellations of causes.

The main causal model used by epidemiologists is Rothman’s “pies” [5]. The idea is that a sufficient causal complex (a pie) is represented by the combination of several component causes (slices of the pie). A set of component causes occurring together may complete the “pie”, creating a sufficient cause and thus initiating the disease process. Rothman’s model has been useful on several accounts. For example, suppose three factors, A, B and C make a sufficient cause of disease X. Then, one can see that A will appear to be a stronger or weaker cause depending on how common are the other “slices” B and C. A will have a big impact on disease in a population in which B and C are common, but no effect at all (through this sufficient cause) where B or C is absent. If it were true that the sufficient cause A+B+C were the *only* pathway to disease X, then it would follow that blocking or eliminating any of these three factors would prevent the disease. Thus A and B and C would be *necessary* component causes. But if A for example also contributed to a sufficient cause with factors D, E and F, then blocking B would not prevent disease X. This more complex view (many pies to which factors contribute) is supported by the epidemiologic evidence for most chronic diseases: there are few examples of necessary component causes for cancer or heart disease.

The foregoing discussion concerns our understanding of disease causality at the *individual level*. The model looks different if we shift from the individual to the *population*. Here, the idea of

single “necessary” components makes sense. If we consider the current epidemic of lung cancer, for example, there is no doubt that it is attributable to the diffusion of the habit of smoking. For, although we cannot attribute any single case of lung cancer to that individual’s smoking habits, there is no doubt that on a population level the epidemic would not have occurred without cigarette smoking. Notice that this assertion is not contradicted by the fact that lung cancer does occur among nonsmokers. Indeed, the evidence for smoking as a (population level) cause of lung cancer is quite strong: the risk of cancer in those who stop smoking decreases considerably in comparison with continuing smokers, and after a few years approaches the risk of non-smokers [6]. It should be clear then, that we have to apply different criteria of causation when considering the causes of disease at the individual or population level. We can say that for chronic diseases, the model of causal complexes in which there are necessary components is valid at the population level.

Another difficulty with Rothman’s pies is that they explain the combination of component causes in a sufficient complex, but do not tackle the temporal sequence at all, an aspect that has been addressed by more recent models.

## **1.2. Identifying intermediate variables**

After Rothman’s model, other proposals have been put forward in epidemiology. At least three deserve being mentioned: graphical models (as e.g. in Pearl’s approach) (7), counterfactual models (8) and structural equation models (9). These models are worth mentioning because they have added some layers of complexity to the discussion on causality, and have also contributed to solving some outstanding issues, including a more sophisticated approach to confounding.

One of the main challenges to correctly identifying causal sequences involving intermediate variables (like biomarkers) is knowing whether the “intermediate” variable belongs to the causal pathway between exposure and disease, or whether it lies on a separate pathway, correlated in some way with exposure or disease. Explanations for the biomarker’s association can include confounding, since the epidemiologist’s view of the process is always a population perspective. For example, it is likely that certain mutations are genuine intermediate markers in causal pathways between certain chemicals and cancers, whereas other mutations are a consequence of a different chain of events, like genomic instability that arises in cancer cells (i.e. an effect of disease, not a cause). As an example of probable confounding, it has been shown that C-Reactive Protein (CRP) levels change with changing levels of other markers of inflammation and with levels of exposure to environmental risk factors for heart disease [10]. It is not clear whether CRP itself lies within the causal pathway or is only a confounded marker for other changes. The distinction is of critical importance in epidemiology: if the biomarker is a confounder, then its effect should be controlled to

produce less biased estimates of associations in the web. If however, the biomarker is on the causal pathway, then controlling for it will *introduce* bias, of potentially substantial magnitude.

Very often intermediate events are both causal events *and* confounders. For example, the development of respiratory disease (measured for example by a change in forced expiratory volume in the first second (FEV<sub>1</sub>)) is an independent predictor of both mortality and subsequent weight loss, and is influenced by prior weight gain (11).

One statistical approach to disentangling confounding uses structural equation models based on the logic of counterfactuals (8). The basic idea is that the exposure leading to changes in the intermediate marker could be theoretically randomized, to create the counterfactual instance in which those with the marker and those without have exactly the same levels of exposure. This would enable us to distinguish a genuine intermediate marker (e.g. CRP) from one that is confounded by exposure. This is typically only a “thought experiment” because most exposures cannot be randomized, except for certain preventive or therapeutic interventions. In the absence of real randomization, the approach involves creating a system of equations which, under certain assumptions, can estimate the counterfactual set of outcomes that each subject would have experienced if (s)he had experienced exposures other than the one actually received.

A similar approach founded on counterfactuals uses graphical methods (7). According to Pearl, a causal graph “is a directed acyclic graph (DAG) in which the vertices (nodes) of the graph represent variables and the directed edges (arrows) represent direct causal effects” (Figure 1 is an example). The main objective of DAGs is to separate the language of statistical association from the language of causality, by making the latter explicit in a graphical form. According to Pearl, the statistical language does not permit us to distinguish between statistical dependence, quantified by conditional probabilities, from causal dependence, “for which we have no expression in standard probability calculus”. The first to apply this approach was the geneticist Sewall Wright, who noticed that equations are symmetrical objects, i.e. they can be rewritten in order to exchange the dependent and the independent variables; therefore Wright complemented equations with a “path diagram” [7].

The notation of DAGs has the merit of making causal assumptions explicit; through the *separation* of different pathways in the graphical structure, it is possible to simulate an experiment (even in the absence of randomization) and thus separate causality from confounding. The effect is defined as “the capacity to transmit changes among variables”. As Figure 1 shows, if we separate Z from X we can evaluate whether X is a genuine causal factor or if it is confounded by Z. The joint distribution associated with the modified model describes the post-intervention distribution of variables (i.e. the controlled or experimental distribution): if X represents a treatment variable, Y a

response variable and  $Z$  some covariate that affects the amount of treatment received, the post-intervention distribution assesses treatment efficacy by comparing aspects of the distribution at different levels of  $X$ .

Once the paths are clarified and separated, one can write a series of equations which describe them, and use these equations to estimate the indicated associations. These equations will be valid under two assumptions: (a) the graph is acyclic, and (b) all the error terms are jointly independent. Both assumptions are frequently violated in biomarker research: (a) very often feedback, i.e. circularity, is encountered (e.g. in the example of obesity, which causes cardiovascular disease, which in turn leads to weight loss), and (b) errors are not independent. But when conditions (a) and (b) are met, we can predict post-intervention distributions from pre-intervention distributions even in the absence of real intervention, i.e. of a randomized trial.

## **2. Epidemiology and the interaction of causes**

There has been a long discussion on “interactions” in the literature of chronic disease epidemiology. Unfortunately, many of these papers address statistical issues such as the type (additive or multiplicative) of the joint effect of variables, providing very little insight into the underlying (biologic) mechanisms that could justify the choice of a model. Pathophysiologic mechanisms for important chronic diseases are of course complex and for the most part poorly understood, but one general principle is very likely to hold across many pathways: the interaction among the component causes occurs dynamically, over time. The onset of disease involves an essential temporal sequence, and this fact has rarely been dealt with in epidemiologic considerations of interaction [12]. Exposure effects may be very different when the exposure acts upon a population whose members are at different stages along a causal pathway, and when those stages are not known to the researcher. This will appear as heterogeneity (potentially quite severe) in responsiveness when viewed statically at a single point in time, as when epidemiologists look backwards from the moment of incidence and compare the exposure histories of cohort members.

What we are saying is that there are two fundamental types of methodological inadequacy in the epidemiologic investigation of interaction. One derives from simple ignorance of the underlying biological processes. The second, and the one typically addressed by epidemiologists, has to do with measurement problems: mismeasurement of variables, the impact of misclassification, and the lack of power of most studies focused on the investigation of interaction (13-15). We believe that the first is likely to be much more important, although it has received less attention.

### **2.1. A taxonomy of gene-environment interactions**

It is likely that there are almost always genetic component causes of any disease, even those (like lung cancer) that also have important environmental causes. Thus it is particularly important to understand how genetic and environmental factors may contribute to the same sufficient causes, or more generally, how they interact.

An effective taxonomy to describe gene-environment interactions (GEI) (irrespective of their being additive or multiplicative) has been provided by Ottman [16] (Figure 2). This is quite useful because it is based on concrete biologic knowledge. In Ottman's Model A, the effect of G (genotype) is to produce or increase expression of a risk factor (E) that can also be produced (or perhaps eliminated) nongenetically. For example, homozygotes for the phenylketonuria (PKU) mutation have a deficiency in the enzyme required to convert phenylalanine to tyrosine. If untreated, they will accumulate phenylalanine in the blood and develop mental retardation, but careful dietary restriction can keep phenylalanine concentrations low, and prevents retardation. In Model B, G exacerbates the effect of E. For example, Xeroderma Pigmentosum is an autosomal recessive disorder in which exposure to ultraviolet (UV) light causes a large number of skin cancers because of a defect of DNA repair enzymes. However, skin cancer is associated with UV exposure also in people without XP. In Model C, E exacerbates the effect of G but there is no effect in persons with the low-risk genotype. For example, an autosomal dominant disorder, porphyria variegata, is characterized by severe skin problems. Exposure to barbiturates strongly exacerbates the symptoms and can lead to death. In Model D, both G and E are required to obtain the effect. G6PD is an X-linked recessive disorder: individuals are asymptomatic unless they eat fava beans; in which case they develop severe hemolytic anemia. Fava beans do not produce any symptoms in normal individuals. Finally in Model E, G and E both have separate effects, but when they occur together the effect is much greater. For example, COPD risk is increased in smokers without alpha-1-antitrypsin deficiency and in nonsmokers with the deficiency, but risk is increased greatly in smokers with the deficiency. We suspect that most gene-environment interactions relevant to environmental exposures and common chronic diseases belong to category E.

## **2.2 Interaction, additivity and the limitations of statistical models**

Epidemiologists of chronic diseases have discussed for decades whether different external exposures are likely to show their effects in an additive or a multiplicative manner, i.e. whether the joint effect of two or more exposures (or of a genetic factor and an environmental one) is the sum of their separate effects or their product, or something else (13). Resolution of this issue has been difficult, partly because of a lack of high quality data from sufficiently large studies, and partly because of the difficulty of distinguishing underlying biologic behaviors from their statistical

models. Depending on the statistical models used, a certain joint estimate will suggest an underlying biologic interaction, or biologic independence. Here again it is important to notice that the biologic behavior (how different component causes combine) occurs within individuals, while the statistical models evaluate population data. There is still much to learn about how to make inferences across these levels.

The necessary biostatistical models for evaluating interaction – usually multivariate regression models -- are based on the analysis of variance. This is easiest to see in the case of the ordinary least squares multiple regression model, but it is true as well for the epidemiologist's preferred tools – the logistic, Poisson and Cox regression models. These all estimate parameters by quantifying (or partitioning) the amount of the variation in risk that should be attributed to one or another independent covariate. In structure, these models are generally linear – thus implicitly assuming that the “main effects” of two or more environmental exposures, or of several genetic and environmental factors, will combine additively in affecting disease. Variances are computed, and the role of the two main effects (or their interaction) is apportioned accordingly. But Lewontin argues that the analysis of variance approach is often misleading [17]. There is no theoretical justification for the presumption of a linear explanation (this is done for the sake of simplicity but is not generally based on any formal biologic assumptions). In an essay entitled “The Analysis of Variance and the Analysis of Causes”, Lewontin cites experimental data to argue that mutations often cause a change in what is called the “norm of reaction”, i.e. the ability of the organism to react to different environmental conditions. The way in which the mutant strain will react, say, to different temperatures, is not consistent or predictable across the range of varying environmental conditions. What this suggests is that a non-linear model may be needed to describe the interaction between a change in genotype and a change in environmental conditions. The key point is that the analysis of variance will correctly correspond to an “analysis of causes” (i.e. quantifying the relative importance of the main effects of genes, environment and their interactions) only when: (a) environmental exposure-response relationships are linear for individuals with each of the different genetic polymorphisms, and (b) the study includes a sufficiently broad range of exposures to provide statistical power to detect an interaction. Reviewing Ottman's five models, the first of these 2 conditions will only hold for models D and E. Thus, in the absence of considerable knowledge of biological mechanisms of disease and the roles of environmental exposures and gene polymorphisms, it will generally be inadvisable to use standard statistical models, apportioning variance, to evaluate gene-environment interactions.

### **3. Apportioning cause: attributable fractions**

One of the principle motivations for sorting out the various components of a causal web is so that we can make predictions about how much disease would be prevented if a certain factor were blocked or eliminated from the web. There is a large literature in epidemiology on the calculation of various statistics for this purpose, often called attributable fractions [18]. Greenland and Robins (1988) have shown that the quantity that is typically estimated by epidemiologists (which they call the excess fraction), is different from (and in most cases less than) the etiologic fraction – that proportion of the disease burden that is causally related to the exposure [19]. The standard methods will not generally estimate the proportion of cases which are etiologically related to an exposure; generally they will underestimate this quantity, by an unknown amount. There are several limitations of the standard methods, but the most important is that the usual formulas cannot account for the possibility that an exposure may move forward in time the onset of a case that would have occurred eventually, in the absence of exposure.

Suppose we are interested in estimating the contribution of a workplace asthmagen to the rate of asthma in an occupationally exposed cohort. Without strong biologic assumptions, it is not possible to say whether there were new cases of asthma in the study period that would have occurred in the absence of the exposure, but whose time of onset was advanced by the exposure. Including this kind of "etiologic case" (as Greenland and Robins call it) in the total burden of the exposure seems appropriate, but cannot be done without strong biologic assumptions. As with the challenge of studying interaction, this caution on the interpretation of attributable fractions stems from the limited understanding that epidemiologists have of how to study the temporal dynamics of causal processes.

#### **4. Main effects, interactions and the absent-minded Mr. Smith**

Epidemiologists tend to focus primarily on the “main effects” of single exposures when analyzing the role of the environment in causing disease [20]. By “main effect” we mean the fact that the contrast of interest is between those exposed to a single environmental agent and those unexposed, irrespective of other exposures or genetic variations. Interactions are considered to be something secondary, if not an interference, to the direct and (unicausal) association of interest.

The “main effects first” strategy may appear parsimonious, but it is inconsistent with what we know about the mechanisms of carcinogenesis and other chronic diseases, and also with common-sense reasoning about causality. For, in carcinogenesis it is well established that the pathway to a tumor includes several stages, and that some exposures can lead to cancer by “completing” the causal chain already initiated by previous exposures. This implies a lack of independence between “earlier” and “later” causes that would seem to conflict with an approach

which views interactions as of secondary importance. In general, we are probably constantly affected by “incomplete” causal chains, *which can be precipitated by timely causal events.*

#### 4.1 Mr. Smith’s house on fire

Here is a commonsense example which may help to illustrate why interactions cannot be secondary phenomena in any causal process consisting of a chain of steps. Let us suppose that Mr Smith is quite absent-minded, so that he often leaves the gas oven in his kitchen alight. Let us also suppose that his house is equipped with a fire alarm; and if this is working, let us say that, for the sake of simplicity, fire fighters will always arrive and extinguish any fire. If, however, the gas is alight and the alarm is not functioning, the probability of a fire is 1 (100%).

There are several scenarios that we can imagine. To start, we establish some “a priori” probabilities of certain events occurring in an interval of time, say one day:

- A. the probability that Mr Smith leaves the gas alight is 50%, or  $p(A) = 0.5$
- B. the probability that the alarm system does not work is 1%, or  $p(B) = 0.01$
- C. the probability that a fire develops for reasons other than those considered here (the “background risk”) is 1/1,000, or  $p(\text{not } A \text{ and not } B) = p(C) = 0.001$

With these assumptions, we can easily calculate the risk of fire under various scenarios:

1. The scenario of ignorance. If Mr Smith does not remember whether he left the gas on, and he does not know if the alarm works, then the probability of a fire occurring through the causal chain involving these two factors is:

$$p(A \text{ and } B) - p(\text{not } A \text{ and not } B) = (0.5 \times 0.01) - 0.001 = 0.005 - 0.001 = 0.004.$$

This figure is analogous to an attributable risk, as it expresses the probability of the event occurring through some specific mechanism or causal chain. The *relative* risk of a fire occurring through this chain, compared to the risk of fire through some other causal chain (C, the “background risk”) is  $0.005/0.001=5$ .

2. A scenario of partial knowledge. If Mr Smith knows that he left the gas on but he does not know if the alarm works, then the probability of a fire is:

$$p(B \text{ given } A) - p(\text{non-}A \text{ and non-}B) = 0.01 - 0.001 = 0.009.$$

The relative risk for this causal chain compared to the background risk is  $0.01/0.001 = 10$ .

3. The scenario of perfect knowledge. If Mr Smith knows both that he left the gas on AND that the alarm does not work, then the probability of a fire is 1; the probability that the fire arises as a consequence of this particular causal chain is  $1 - 0.001$ , and the relative risk is  $1/0.001 = 1000$ .

In all of these simple calculations, we assume, for the sake of simplicity, the independence of A and B, i.e. absent-mindedness has nothing to do with malfunctioning of the alarm. Although

this example is overly simplistic, it has relevance to the problem of attributing cancers to particular causes. We know that cancer requires several stages to develop, and we can imagine that some of the exposures that lead to cancer are common (around 50%, like cigarette smoke) and others rare (like some genetic traits). However, what really counts is their combination, and in particular the fact that some exposures can “complete an incomplete causal chain”. What makes this insight particularly important for the problem of attributing causes of cancer (or any other disease) is that while we are confident that multiple factors act through causal chains such as these, we are almost always quite ignorant about what components make up these chains, whether they must act in a particular temporal order, and so on.

Returning to the house fire example, notice the impact of knowledge on the relative risk:

1. In the scenario of ignorance, in which we did not know whether the gas had been left on, nor whether the alarm would function, we obtained a relative risk of 5 comparing this causal chain to the background risk.
2. If we had partial knowledge – for example we knew that the gas was on, but not whether the alarm would function, the relative risk increased to 10.
3. Finally, if we also knew that the alarm was broken, then we could be certain that a fire would develop.

The relevance of these arguments to the previous discussion is that they suggest that interaction here is not a secondary property that can only be expressed according to some mathematical (additive or multiplicative) model, but it is simply the ability to complete an incomplete causal chain. The fact that we assumed that absent-mindedness had nothing to do with whether the alarm would work (statistical independence of the two “risk factors”) did not diminish the fundamental reality of the interaction between these two causal factors. In simplest terms, leaving the gas on and the fire alarm failing were independent phenomena, and yet they clearly interacted to cause a house fire.

We can summarize the epidemiologic lessons from the absent-minded Mr. Smith, in two points: (a) a pathogenic process can reach a stage at which even an unlikely exposure (with low prevalence) becomes extremely powerful in triggering the final transformation, if the subject has already undergone most of the required stages; and (b) *a priori* knowledge of susceptibility can strongly modify our predictive ability. An important implication of the latter point is that ignorance of the genetic components of a necessary cause leads to lower estimates of the magnitude of the risk from an environmental cause (and vice versa). Epidemiologists are well-aware that poor characterization of an exposure often leads to underestimation of its risk – our point is that poor

characterization of entirely distinct components of the same sufficient cause will *also* lead to underestimation of a risk or even failure to detect the risk entirely.

## **5. Causal chains, heredity and acquired susceptibility**

Epidemiologists in recent decades have turned their attention to the search for gene-environment interactions, or (a related concept) genetic susceptibility [21]. While some important findings have been reported, there have been as yet few examples of dramatic differences in genetic susceptibility to environmental agents. Instead, there are a steadily growing number of reports of modest impacts on environmentally-induced disease [22]. *Acquired* susceptibility has not received the same degree of attention as hereditary susceptibility, but there is increasing evidence that, in carcinogenesis, the former (e.g. mutations relevant to carcinogenesis) is common even at birth and in the first years of life. We will try to show how acquired susceptibility adds further complexity to the idea of gene environment interaction; making it all the more important for epidemiologists to understand the causal chains they study.

Before summarizing some recent studies on acquired susceptibility to cancer, it is important to be clear about the distinction between two similar-sounding concepts: heritability and genetic causation or determinism. Heritability has to do with similar patterns of observable traits between parents and offspring, while a characteristic is “genetically determined” if it is coded in and caused by the genes in a normal environment. Two extreme examples may help to clarify the distinction. The number of fingers in humans is totally genetically determined; the rare deviations from 5 fingers on each hand being caused by defects of development – from thalidomide for example, and therefore are not heritable. In contrast, wearing skirts among European populations has a very strong heritability (it occurs only in women, with the exception of the odd Scotsman). Skirt wearing is thus closely related to having two X chromosomes, but it is not genetically determined (23). Such misconceptions are clearly relevant to the discussion about the heritability versus genetic determination of cancer. For example, the study of disease clustering in identical twins does not provide clear evidence with which to infer that cancer (or schizophrenia for that matter) is due to inherited changes in DNA. Identical twins often inherit similar environments from their parents. The same applies to claims that IQ has 60% heritability, academic performance 50% and occupational status 40%: these figures do not mean that such characteristics are inherited through genes (DNA), i.e. that there is genetic determination, but only that there is a strong association between the characteristic in children and their parents.

### **5.1. Gene Mutations and Environmental Exposures: acquired susceptibility to cancer**

One approach to studying gene-environment interactions evaluates cancer risk from exposures to carcinogens in people who have mutations shown experimentally to play a role in carcinogenesis *in vitro* or in an animal model. If such a mutation is environmentally induced, and if it increases cancer risk in humans, then this would represent a type of genetic susceptibility not from a fixed trait, but rather acquired from an environmental exposure.

#### Mutations at birth.

Mutations can arise very early in life. A striking recent observation was the finding of a very high proportion, in healthy newborns, of fusion genes TEL-AML1 and AML-ETO associated with lymphocytic leukaemia (24). The frequency of these mutations in healthy newborns was about 100 times higher than the expected incidence of lymphocytic leukaemia; implying that this mutation, detected at birth, may be an early step in a causal chain leading to that disease. While the origin of such mutations is not known – but could reflect *in utero* exposure to genotoxicants – it is clear that these mutations alone insufficient to explain the onset of leukemia, which probably requires further “hits” to the precursor cells. In another investigation in humans, Finette et al (25) found a high prevalence of *hprt* mutations at birth in healthy children, coming to similar conclusions as Mori and colleagues.

A number of other studies have identified specific mutations caused by environmental chemicals, even early in life. These mutations may or may not constitute acquired susceptibility, but they provide evidence that such effects may be identified in the future. In a series of well-designed experiments, Somers et al (26) reported increased mutation rates in herring gulls and mice exposed to air pollution at levels that characterize normal urban environments. In mice, in fact, mutations were transmitted transgenerationally, i.e. they were attributed to DNA damage in sperm cells. Somatic mutations in newborns have been related to air pollutants (27), and mutations in germ cells have been attributed to air pollution or cigarette smoking (28, 29). In a further study, human mother-newborn pairs exposed to high levels of indoor pollution from coal smoke were investigated (30). For all markers, including DNA adducts, newborns had levels which were higher than in the mothers, although transplacental exposure levels were 10-times lower than the paired mother exposures. In one experiment, pregnant rats were exposed to environmental tobacco smoke; 8-OH-dG adducts were formed in the fetal kidney, liver and brain, with increases that were similar to the risk of human cancer related to ETS. The distribution in different organs depended on gestational stage (31). In summary, these findings suggest that mutations can be already present at birth, predisposing to cancer if further hits occur.

#### Age Effects on Risk.

A further aspect of acquired susceptibility to cancer is the possibility that newborns may be particularly susceptible to carcinogens. In revising cancer risk assessment guidelines, the U.S. Environmental Protection Agency (EPA) analyzed animal cancer bioassay data over different periods of life (32). Results indicated a 5- to 60-fold increased carcinogenic sensitivity in the birth-weaning period per unit dose (defined as mass/body weight<sup>0.75</sup> - day) for mutagenic carcinogens and a somewhat smaller increase--centered about 5-fold--for radiation carcinogenesis per Gray. The authors found a similar increased sensitivity in the fetal period for direct-acting nitrosoureas, but no such increased fetal sensitivity was detected for carcinogens requiring metabolic activation. Radiation experiments indicated that carcinogenic sensitivity is not constant through the "adult" period, but the dosage delivered in 12- to 21-month-old animals appears a few-fold less effective than the comparable dosage delivered in young adults (90-105 days of age).

The example of age is somewhat different from that of acquired susceptibility in subgroups, but it is relevant because early exposure can be a mechanism by which highly susceptible groups arise in the population.

## **6. Conclusions**

In this paper we have explored different models for the explanation of cause in the epidemiology of disease arising out of genes, environments and the interplay between environments and genes. We have discussed some of the challenges that still face epidemiologists as they try to disentangle the contributions of multiple risk factors in chronic disease. Interaction is a fundamental characteristic of any causal process involving a series of probabilistic steps, making it very difficult to estimate the individual contribution of any single factor in a causal chain. We conclude that it is vitally important for epidemiologic research to study "interactions", and in particular acquired susceptibility to disease through the use of appropriate models of causation.

Epidemiologists should continue to search for gene-environment interactions in the causation of chronic diseases, but the hunt will be slow; ignorance about steps in a causal chain will hamper the identification of component causes, either environmental or genetic, in that chain.

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Figure 1. An example of DAG (see text). From reference 7. The letters indicate “nodes” in the graph and stand for variables in the causal model. Arrows (“edges”) represent relationships. Unobserved exogenous variables are connected by dashed arrows.



**Figure 2. Ottman's taxonomy of gene-environment interactions (G=genotype, E=environment)**

**Model A: the effect of G is to produce or increase expression of a risk factor (E) than can also be produced nongenetically (e.g. PKU)**

**Model B: G exacerbates the effect of E (e.g. UV and skin cancer)**

**Model C: E exacerbates the effect of G but there is no effect in persons with the low-risk genotype (e.g. porphyria variegata)**

**Model D: both G and E are required to obtain the effect (e.g. G6PD deficiency)**

**Model E: G and E both have a separate effect, but when they occur together the effect is much higher (e.g. alpha-1-antitrypsin and COPD)**