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Gene expression in teratogenic exposures: A new approach to understanding individual risk

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ABSTRACT

The phenomenon of partial or incomplete penetrance is common to many paradigms of exposure to teratogens, where only some of the exposed individuals exhibit developmental defects. We here argue that the most widely used experimental approaches in reproductive toxicology do not take partial penetrance into account, and are thus likely to miss differences between affected and unaffected individuals that contribute to susceptibility for teratogenesis. We propose that focus on the variation between exposed individuals could help to discover factors that may play a causative role for abnormal developmental processes that occur with incomplete penetrance.

1. The problem

Agents with developmental toxicity often cause defects or anomalies only in a fraction of the exposed individuals. Dose, time and duration of exposure, as well as biological features of the affected tissues themselves are all thought to influence developmental outcomes, such as the risk for neural tube defects [1] or long-term adverse health outcomes, such as metabolic syndrome and cardiovascular disease [2]. Susceptibility of an individual to the environmental exposure is generally believed to be determined by pre-existing genetic factors [3]. However, even in genetically identical animals, such as highly inbred strains, not all animals respond in identical fashion in many exposure paradigms. In fact, often only the minority of the exposed individuals are affected, while a large group of animals with the same exposure develop or function normally. Thus, the phenotypic outcome presents with what in genetics terms would be called "incomplete penetrance" or "partial penetrance".

Examples for phenotypes of incomplete penetrance after teratogen exposure are the heart defects and neural tube defects that occur in diabetic pregnancies in inbred mice. Although these defects are strikingly more frequent in mice that are hyperglycemic, not all progeny within the same litter exhibit defects, and some of the litters from a group of experimental pregnancies may even be unaffected. Furthermore, maternal diet can affect penetrance of neural tube defects in embryos that develop in diabetic FVB females [4]. Genetic factors also appear to play a role in penetrance, as in diabetic pregnancies embryonic defects are less frequent in the inbred C67BL/6 strain than in the inbred strain FVB [5], and considerably more frequent in the inbred non-obese diabetic (NOD) strain [6] (and Salbaum et al., unpublished results), an inbred mouse line with spontaneous diabetes [7]. Thus, diabetic pregnancy in inbred strains is an ideal model to dissect genetic and environmental contributions to teratogen-induced partial penetrance of anomalies.

From early genetic studies, it was proposed that partial penetrance of phenotypes reflects a threshold phenomenon [8,9], based on Grüneberg's statement that developmental defects "*are 'quasi-continuous' characters in the sense that the underlying . . . basis is a continuous variable (generally not yet identified). . . which is divided by a physiological threshold into normal and abnormal animals. . .*" [9]. In this model, the continuous variable is conceptualized as

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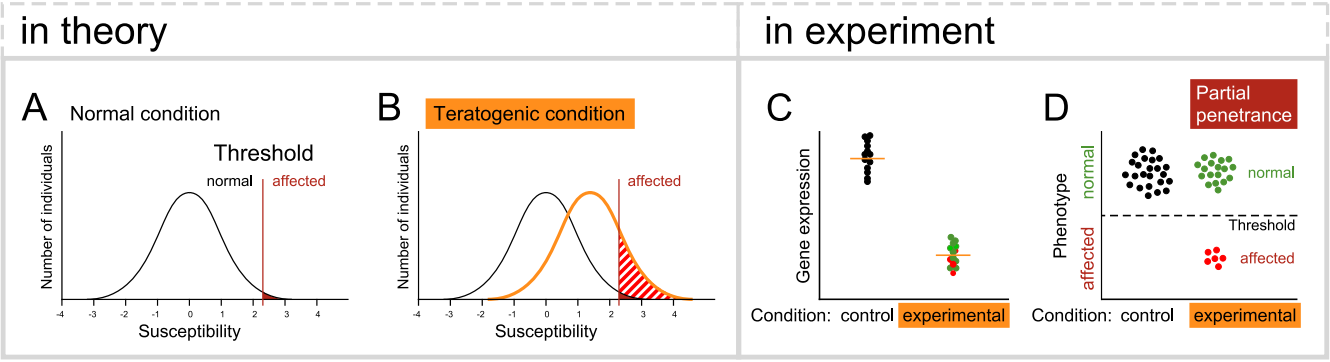

Fig. 1. Paradigms with partial penetrance: incongruity between approach and outcome. (A) In normal conditions, almost all individuals in the population fulfill the required threshold(s) and very few animals ever exhibit the abnormal phenotype, hence the small fraction of affected individuals (red). Phenotype penetrance is close to 0%. (B) The teratogenic exposure (orange curve) moves the mean of the distribution in a direction that increases the number of affected individual in the population of all exposed individuals. The fraction of affected individuals (large red hatched area) determines the penetrance, which, in the extreme, could reach 100%. (C) Traditional approaches in gene expression profiling identify differentially expressed genes by virtue of a statistically significant difference between the mean of a control group's gene expression values (black) and the mean of the gene expression values for a group of experimental samples (green and red). For simplicity, only one direction of change is depicted here, namely lower mean expression levels in the exposed group; an analogous situation would be present when the expression levels for a given gene would be higher in experimental samples than in controls. (D) The typical outcome from exposure to the teratogenic condition (orange) is that a fraction of individuals exhibits the abnormal phenotype (red) while other exposed individuals undergo normal development (green). The dashed line represents the threshold between normal and abnormal development. It is visually obvious that the discovery paradigm in C cannot resolve molecular differences between individuals with normal and abnormal phenotype, because the mean for the experimental group includes normal and affected individuals. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

a normal distribution of data points for all individuals within a population. Individuals at the extreme fail to fulfill the requirements for proper development and exhibit a defect (Fig. 1A), which – in wildtype animals – would be a very rare occurrence. Increased risk for adverse outcomes from teratogen or toxicant exposure then results from a shift in the mean of the distribution, increasing the fraction of individuals surpassing the threshold (Fig. 1B). Although this model was originally formulated for categorical phenotypes (in Grüneberg's studies skeletal defects were scored as "present" or "absent"), the general considerations also apply to quantitative outcomes, or phenotypes with variable expressivity: then the extremes of the distribution correspond to the "mildest" and "severest" manifestation, or lowest and highest measurements for a given outcome parameter. The paramount goal in molecular reproductive toxicology is to identify the "*not yet identified*" continuous variable(s) that underlie the distribution, and that ultimately determine risk for adverse outcomes.

Many laboratories, including our own, have turned to unbiased approaches, such as microarray-based or sequencing-based gene expression profiling, to identify genes, molecules and pathways that are targeted in the teratogenic exposure, and that might explain detrimental outcomes. The standard approach is to compare profiles from unexposed control animals, or embryos from unexposed pregnant dams, to profiles from experimental animals or embryos that experienced the exposure conditions. However, in paradigms with incomplete penetrance, this approach may be inappropriate on both theoretical and empirical grounds, as we will argue below. Thus, we have to ask ourselves: have we been looking for the right thing? Have we missed something important?

The conventional approach to the interpretation of gene expression data is to search for consistent differences between a control group and an experimental group (Fig. 1C) that fulfill specific statistical criteria. However, these approaches can only reveal changes that affect all (or most) samples in the experimental group, and thus cannot account for phenotypic outcomes that affect only a (minor) fraction of the individuals, such as in the case of partial penetrance (Fig. 1D). Thus, it could be argued that, while changes in all exposed animals confer some vulnerability relative to a specific outcome, the actual pathogenic triggers are present only in some exposed individuals, those at the extremes of a distribution that manifest with the abnormal phenotype or adverse outcome.

2. A new alternative

We propose that the traditional concept omitted an important second mechanism that can increase risk for abnormal phenotype: a greater fraction of affected individuals could also result from greater variance in the distribution (Fig. 2A). In this scenario, the overall mean remains unchanged, but more individuals fall outside of the threshold. Thus, the net effect is the same as in Fig. 1B, placing a larger number of individuals at risk for abnormal phenotype, and the same pattern of phenotypic outcomes would be achieved as in Fig. 1D. However, the conventional frameworks of interpretation for gene expression data do not consider variability as an independent parameter. In fact, they seek to minimize it, through pooling of several individual-derived samples [10], or in the statistical framework [11–13]. Consequently, the concept of increasing variability has not been applied at the experimental level.

As increasing variability of expression levels is reflected in a wider distribution curve, the mean of the wider distribution can be similar or different from the control group (Fig. 2B). In either scenario it is intuitively obvious that increasing variability can account for incomplete penetrance [14–16] (when the differential gene expression scenario in Fig. 1C cannot). Furthermore, if variability is increased for multiple parameters, such as two or more genes – in the same animal – the individual liability for abnormal development would increase. Experimental strategies pursued so far have not considered this at a systems-wide level. Therefore, in contrast to traditional approaches that all seek to minimize variation, we propose that focus on variability of gene expression would uncover new genes, pathways and mechanisms that are involved in abnormal development.

To illustrate how our concept impacts gene discovery and interpretation of gene expression profiling data, we offer two experimental examples: microarray-based genome-wide expression surveys from our chemical model of type I diabetes induction by injection of Streptozotocinin the FVB mouse strain (as published previously [17]), and from the non-obese diabetic mouse strain [6],