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Environmental Epigenetics

Valentina Bollati¹ and Andrea Baccarelli^{1,2,*}

¹Center of Molecular and Genetic Epidemiology, Department of Environmental and Occupational Health, Università degli Studi di Milano and Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena, Via San Barnaba 8, Milan 20122, Italy.

²Exposure, Epidemiology and Risk Program, Department of Environmental Health, Harvard School of Public Health, Boston, Massachusetts, USA.

Abstract

Purpose of the review—Epigenetics investigates heritable changes in gene expression occurring without changes in DNA sequence. Several epigenetic mechanisms, including DNA methylation and histone modifications, can change genome function under exogenous influence. We review current evidence indicating that epigenetic alterations mediate effects from exposure to environmental toxicants.

Recent findings—Results from animal models indicate that in-utero or early-life environmental exposures produce effects that can be inherited transgenerationally and are accompanied by epigenetic alterations. The search for human equivalents of the epigenetic mechanisms identified in animal models is under way. Recent investigations have identified a number of environmental toxicants that cause altered methylation of human repetitive elements or genes. Some exposures can alter epigenetic states and the same and/or similar epigenetic alterations can be found in patients with the disease of concern. Based on current evidence, we propose possible models for the interplay between environmental exposures and the human epigenome.

Summary—Several investigations have examined the relation between exposure to environmental chemicals and epigenetics, and identified toxicants that modify epigenetic states. Whether environmental exposures have transgenerational epigenetic effects in humans remains to be elucidated. In spite of the current limitations, available evidence supports the concept that epigenetics holds substantial potential for furthering our understanding of the molecular mechanisms of environmental toxicants, as well as for predicting health-related risks due to conditions of environmental exposure and individual susceptibility.

Keywords

Epigenetics; DNA methylation; Histone modifications; Environmental exposures; Transgenerational effects

Epigenetic Mechanisms

Epigenetics is the study of heritable changes in gene expression that occur without changes in DNA sequence (Wolffe and Guschin, 2000). Epigenetic mechanisms are flexible genomic parameters that can change genome function under exogenous influence, but also provide a mechanism that allows for the stable propagation of gene activity states from one generation

*Corresponding author Andrea Baccarelli, MD PhD Center of Molecular and Genetic Epidemiology Department of Environmental and Occupational Health Università degli Studi di Milano and Fondazione IRCCS Ospedale Maggiore Policlinico, Mangiagalli e Regina Elena Via San Barnaba 8 20122 Milan, Italy Tel +39 02 503 20145 Fax +39 02 503 20103 andrea.baccarelli@unimi.it.

of cells to the next. There are at least two kinds of epigenetic information that can be inherited with chromosomes. The first is DNA methylation, the second involves changes in chromatin proteins, usually due to modifications in histone tails.

DNA methylation

DNA methylation is a covalent modification, heritable by somatic cells after cell division. 5-methyl-cytosine (5MeC) represents 2-5% of all cytosines in mammalian genomes and is found primarily on CpG dinucleotides (Lister *et al*, 2009; Millar *et al*, 2003; Rossella *et al*, 2009).

Cytosine methylation of CpG dinucleotides is found in close proximity to critically important cis-elements within promoters and is often associated with a repressed chromatin state and inhibition of transcription (Orphanides and Reinberg, 2002). DNA methylation also plays an important role in the maintenance of genome integrity by transcriptional silencing of repetitive DNA sequences and endogenous transposons (Bestor, 1998; Hedges and Deininger, 2007).

Histone modifications

Histones can be modified by acetylation, methylation, phosphorylation, glycosylation, sumoylation, and ADP ribosylation (Suganuma and Workman, 2008). The most common modifications are acetylation and methylation of lysine residues in the amino terminal of Histone 3 (H3) and Histone 4 (H4). Increased acetylation induces transcription activation, whereas decreased acetylation usually induces transcription repression. Methylation of histones is associated with either repression or activation of transcription, depending on the lysine residue position (Yan and Boyd, 2006).

Environmental Health and Genes: Beyond Gene-Environment Interactions

According to the World Health Organization (WHO), more than 13 million deaths annually are due to environmental causes and as much as 24% of disease is caused by exposures that can be averted (Prüss-Üstün, 2006). The list of environmental threats to human health includes a large number of environmental pollutants. For instance, in the third National Report on Human Exposure to Environmental Chemicals by the Center for Disease Control and Prevention, 148 different environmental chemicals that can be detected in blood and urine were found in a sample of the U.S. population. The list of pollutants evaluated included metals, phytoestrogens, polycyclic aromatic hydrocarbons, dioxin-like chemicals, polychlorinated biphenyls, phthalates, and several classes of pesticides (Department of Health and Human Services Centers for Disease Control and Prevention, 2005). In Fig. 1, we propose a categorization of how environmental exposures, with particular interest in environmental toxic chemicals, may interact with genetic and epigenetic mechanisms. In particular, we are interested in contrasting genetic vs. epigenetic mechanisms in their possible interplays with environmental exposures.

Gene-environment interactions

The interplay between the environment and human genome has been traditionally presented under the framework of gene-environment interactions (figure 1, path A; also indicated as genotype-environment or GxE interaction) (Baccarelli, 2009; Dempfle *et al*, 2008; Ishibe and Kelsey, 1997; Kraft and Hunter, 2005; London and Romieu, 2009). Under this model, diseases result from interactions between the individual genetic makeup and environmental factors. Geneticists have always held true that the expression of a genetic trait in the phenotype is highly variable, largely depending on the environment to which the individual

carrying the trait of concern is subjected. For instance, in patients with phenylketonuria (PKU), which is caused by mutations to a gene coding the liver enzyme phenylalanine hydroxylase (PAH), the amino acid phenylalanine does not get converted into tyrosine and reaches high levels in blood and other tissues (Scriver, 2007). The elevated phenylalanine levels affect brain development leading to mental retardation. However, a low-phenylalanine diet can keep blood phenylalanine low and avoid the severe effects of PKU. The same concept can be approached from the realm of environmental health: some individuals have low risk of developing a disease as a result of an environmental exposure, while others are much more susceptible. For example, individuals that carry genetic polymorphisms that make their cells less capable to respond to oxidative stress, have been found in several investigations to be more susceptible to the cardiovascular and respiratory effects of air pollution, which produces health effects in humans, at least in part, through oxidative stress generation (Baccarelli *et al*, 2008a; Chahine *et al*, 2007; Park *et al*, 2006).

A purely DNA sequence-based approach (naked DNA snapshot) is not sufficient to fully explain the risks of common diseases, which are modulated by other non-genetic or extra-genetic mechanisms. In fact, growing evidence demonstrates that the molecular influences of the environment extend well beyond the interaction with the DNA sequence. Several investigations, as we will discuss in the following sections, have shown that environmental toxicants modify epigenetic states.

Gene-Environment vs Epigene-Environment

In gene-environment interactions, (Fig 1, path A) the genetic polymorphisms that modify the effects of environmental exposures are transmitted transgenerationally according to Mendelian genetics, and the trait determining effect modifications is generally assumed to follow the same genetic model (dominant, co-dominant, recessive) as that of the levels of expression or function of the protein coded by the locus of concern. A second well-established area of interplay (Fig 1, path B) includes the direct effects of environmental exposures on the genome, e.g., DNA damage and/or mutations induced by environmental exposures. In environmental health, the recognition that exposures could produce DNA mutations represented a major landmark for risk assessment and prevention. Consequently, genotoxic agents have been categorized according to their capability to alter DNA sequence and thus increase disease risk (Siemiatycki *et al*, 2004). Such information has been fundamental to determine environmental risks and shape current regulatory efforts for exposure reduction. In particular potential carcinogenic agents have been carefully tested in in-vitro and in-vivo models of mutagenicity. In human subjects, some of these molecular events may represent early events along the pathways linking carcinogen exposure to cancer. For example, in our own work on the population exposed after the Seveso, Italy accident to high-doses of dioxin (Baccarelli *et al*, 2008b; Pesatori *et al*, 2008), a powerful promoting carcinogen in animals, we showed an increased number of t(14;18) translocations detectable in phenotypically normal blood lymphocytes collected from healthy subjects (Baccarelli *et al*, 2006). This effect may represent an early expansion of lymphocyte clones potentially related with the increased risk of Non-Hodgkin's lymphoma among subjects exposed to high doses of dioxin (Steenland *et al*, 2004). Environmentally-induced DNA mutations can have a transgenerational effect only if occurring in the germline. For instance, parental exposure to ionizing radiation has been shown to increase the frequency of germline mutations detectable in the next generation (Charles, 2001), and confer a predisposition to cancer (Dubrova *et al*, 2000).

In principle, the effect-modification model should apply to epigene-environment interactions as well as to gene-environment interactions. Similarly to the effect modifications demonstrated or postulated for genetic polymorphisms (Fig 1, path A), epigenetic

differences determining disease risk could make individuals less or more vulnerable to environmental insults (Fig 1, path C). However, to best of our knowledge, a formal concept of epigene-environment interaction has not been yet developed and we are not aware of examples of epigene-environment interactions in environmental health or toxicology studies. In environmental studies, the flexibility of epigenetic states has generated a growing interest in evaluating the direct alterations that environmental exposures may produce on epigenetic states (Fig 1, path D), including changes in DNA methylation and histone modifications. Investigations that evaluated alterations in DNA methylation and histone modifications in response to environmental chemical exposures were reviewed by us in a recent article (Baccarelli and Bollati, 2009). Here, we will discuss the biological basis for potential interplays with epigenetic states that might be activated in the presence of environmental exposures and determine health-related effects. We will also discuss whether available evidence suggests that epigenetics provides biological mechanisms for transgenerational environmental effects.

Epigenetic Reprogramming in the Mammalian Development

In mammals, DNA methylation is essential for embryogenesis, during which methylation patterns change dynamically to adapt embryos to be fit for further differentiation (Reik *et al*, 2001). Two main waves of genome-wide epigenetic reprogramming characterize mammalian development that take place at the zygote stage and during primordial germ cells formation (Shi and Wu, 2009).

The genome becomes demethylated during preimplantation, to give rise to a totipotent zygote able to generate any cell type. Active DNA demethylation occurring in the paternal genome shortly after fertilization is independent from DNA replication. On the contrary, the maternal genome remains highly methylated and undergoes passive DNA demethylation following embryo development (Mayer *et al*, 2000; Santos *et al*, 2002). After the first cell cycle, the maternal allele passively loses methylation through cell divisions up to the blastocyst stage (Shi and Wu, 2009). When implantation occurs, DNA methylation levels are then restored by de novo methylation that triggers cell lineage differentiation (Sassone-Corsi, 2002).

The second reprogramming event occurs also during embryogenesis, but only in the primordial germ cells where DNA methylation patterns are erased at all single copy genes and some repetitive elements (Lees-Murdock and Walsh, 2008).

Similar to the pattern of asymmetric DNA methylation in parental genomes, histone H₃K₉ trimethylation and dimethylation exhibit asymmetric modifications in the parental pronuclei (Kurdistani *et al*, 2004; Santos *et al*, 2005; Valls *et al*, 2005; Wang *et al*, 2007; Yoshida *et al*, 2007).

The Dilemma of Epigenetic Inheritance: can Epigenetic Marks Survive Reprogramming?

The cycles of erasure/reprogramming that take place during embryogenesis raise the question about how and how frequently epigenetic marks are inherited transgenerationally, and whether epigenetic inheritance occurs in humans as well as in animal models (Whitelaw and Whitelaw, 2008). Animal experiments provide us with a few examples suggesting that epigenetic marks that are established during the life of an organism can be passed on to the following generations (Probst *et al*, 2009). These include epigenetic states of murine genes associated with distinctive phenotypes such as the agouti locus in the viable yellow (A^{vy/a}) mice (determining fur colour variation between yellow and dark brown), the Axin-fused

(Axin^{Fu}) allele (associated with a kinked tail) (Morgan *et al*, 1999; Rakyan and Whitelaw, 2003) and the Cabp^{IAP} gene, which was identified using a bioinformatic approach and shows sequence homology to the rat CDK5 activator binding protein (Druker *et al*, 2004).

All these models derive their properties from metastable epialleles, which are alleles that are variably expressed due to epigenetic modifications that are established very early during development (Rakyan *et al*, 2002). In metastable epialleles, the epigenetic state can be altered and the alteration shows transgenerational inheritance. Metastable alleles are most often determined by the presence of a retrotransposable element (or retrotransposon). In particular, the murine metastable epialleles A^{Vy}, Axin^{Fu}, and Cabp^{IAP} are all associated with contraoriented insertions of an retrotransposable intra-cisternal A particle (IAP) sequence, a family of retrovirus-like genetic elements coding for virus-like particles (Druker *et al*, 2004; Duhl *et al*, 1994; Vasicek *et al*, 1997).

Epigenetic Inheritance and Metastable Alleles in Animal Experiments: The Viable Yellow Agouti A^{Vy} Mouse

The viable yellow agouti A^{Vy} allele is the most extensively studied murine metastable epiallele. In viable yellow (A^{Vy}/a) mice, transcription originating in a IAP retrotransposon inserted upstream of the agouti gene (A) causes ectopic expression of agouti protein, resulting in yellow fur, obesity, diabetes and increased susceptibility to tumours (Morgan *et al*, 1999). A^{Vy} mice display variable expressivity because they are epigenetic mosaics for activity of the retrotransposon: isogenic A^{Vy} mice have coats that vary in a continuous spectrum from full yellow, through variegated yellow/agouti, to full agouti (pseudoagouti). The distribution of phenotypes among offspring is related to the phenotype of the dam; when an A^{Vy} dam has the agouti phenotype, her offspring is more likely to be agouti. It has been shown that the offspring colour is not the result of a maternally contributed environment, but rather genuinely inherited, and is associated with transmission of a silenced A^{Vy} allele through the female germ line (Morgan *et al*, 1999).

Is there enough Evidence for Epigenetic Inheritance in Humans?

Determining whether equivalents of metastable epialleles exist and are frequent in humans poses substantial challenges. There are just a few reports that have been used to suggest inheritance of epigenetic states in humans. For example, in several cases of familial colorectal cancer, the mismatch repair genes MLH1 and MSH2, which usually exhibit low or no methylation, have been found to be silenced due to promoter methylation, and this has been occasionally detected in successive generations (Chan *et al*, 2006; Hitchins *et al*, 2007; Suter *et al*, 2004). However, these were reports of single families and it has been argued that the promoter methylation identified, even if detected in multiple family members, could be explained by somatic events that occurred after fertilization (Horsthemke, 2007). Whitelaw *et al*. have recently remarked that, in the light of current evidence, the notion that epigenetic marks can be directly inherited across generations in humans remains contentious (Whitelaw and Whitelaw, 2008).

Environmental Influences on Epigenetic States during early Development in Animal Models

Modifications to the environment during early development can lead to permanent changes in the pattern of epigenetic modifications (Fauque *et al*, 2007). Pregnant female rats exposed during time of sex determination to the endocrine disruptor vinclozolin have been shown to exhibit in their male offspring transgenerational disease state leading to spermatogenic defects, prostate disease, kidney disease, immune system abnormalities,

hypercholesterolemia, and an increased rate of tumor development in the F1-F4 generation offspring (Anway *et al*, 2005; Anway *et al*, 2006a; Anway *et al*, 2006b; Anway and Skinner, 2006; Chang *et al*, 2006). Both the F1 generation embryo and F2 generation germ line are directly exposed when an F0 generation pregnant mother is exposed. Therefore, only the F3 generation can provide the first unequivocal signs of transgenerational inheritance. The transgenerational disease states in the vinclozolin F1-F4 generation animals were found to be associated with a transgenerational alteration in the epigenetic programming of the male germ line (Anway *et al*, 2005).

A second example of epigenetic toxicant is represented by bisphenol A (BPA), a high-production-volume chemical used in the manufacture of polycarbonate plastic. In utero or neonatal exposure to BPA is associated with higher body weight, increased breast and prostate cancer, and altered reproductive function. Dolinoy *et al* demonstrated that maternal BPA exposure shifted the coat colour distribution of viable yellow agouti (A^{vy}) mouse offspring toward yellow by decreasing CpG (cytosine-guanine dinucleotide) methylation in the IAP sequence upstream of the Agouti gene (Dolinoy *et al*, 2007a; Waterland, 2009). In addition, CpG methylation was also decreased at the Cabp^{IAP} metastable locus. DNA methylation at the A^{vy} locus was similar in tissues from the three germ layers, suggesting that BPA affected epigenetic patterning during early stem cell development.

Moreover, maternal dietary supplementation, with either methyl donors like folic acid or the phytoestrogen genistein, blunted the DNA hypomethylating effect of BPA (Dolinoy *et al*, 2007a). However, because this investigation was conducted only up to the F2 generation, it did not directly demonstrate inheritance of the epigenetic modifications induced by BPA exposure.

Translating Environmental Epigenetic Effects from Animal Models to Humans

Translating results obtained in mouse models to humans is not straightforward. The agouti model cannot be directly applied to humans, nor is there a human equivalent of kinked tails. One common characteristic of the mouse models described above is that their metastable alleles all include a IAP retrotransposon. IAP sequences are endogenous retrovirus-like mobile elements, present at 1000 copies in the mouse genome. These elements transpose in a replicative manner via an RNA intermediate and its reverse transcription, and their transposition should therefore be tightly controlled by their transcription level (Dupressoir and Heidmann, 1997). In humans, more than one-third of DNA methylation occurs in retrotransposons (Kochanek *et al*, 1993; Schmid, 1998), which represent a large portion of the human genome (Bernstein *et al*, 2006). Among these sequences, Alu and LINE-1 retrotransposons are the most plentiful families representing approximately 30% of the human genome (Babushok and Kazazian, 2007; Grover *et al*, 2004; Kazazian and Goodier, 2002) and are heavily methylated (Yang *et al*, 2004). Because of their high representation throughout the genome, Alu and LINE-1 have been proposed as surrogate markers for estimating global DNA methylation level (Weisenberger *et al*, 2005; Yang *et al*, 2004), but growing evidence indicates that they could have specific and distinct cellular roles (Wallace *et al*, 2008). Hypomethylation of repetitive elements favours their activity as retrotransposable sequences and has been suggested to have deleterious effects on cells, initially through insertional mutations (Kazazian, 2004), and later by introducing genome instability through deletions and genomic rearrangements (Gilbert *et al*, 2002; Ostertag and Kazazian, 2001; Wallace *et al*, 2008).

In healthy populations, inter-individual methylation variations at Alu or LINE-1 elements from blood DNA have also been associated with risk factors for cancer, neurological and

cardiovascular diseases (Baccarelli and Bollati, 2009; Baccarelli *et al*, 2009; Bollati *et al*, 2007; Rusiecki *et al*, 2008). Also the finding of an age-associated decline in repetitive-element methylation in normal tissues of aging individuals suggests a possible role in a variety of common human age-related diseases (Bollati *et al*, 2009).

Epigenetics and environmental toxicants in humans

Exposure to air pollution, particularly to particulate matter (PM), has been associated with increased morbidity and mortality from cardiorespiratory disease, as well as with lung cancer risk (Baccarelli *et al*, 2008c; Brook *et al*, 2004; Peters, 2005; Samet *et al*, 2000; Vineis and Husgafvel-Pursiainen, 2005). In a human study, we recently demonstrated that promoter methylation of the iNOS (inducible Nitric Oxide Synthase) gene was lower in blood samples of foundry workers with well-characterized exposure to PM with aerodynamic diameter < 10 μm (PM₁₀) (Tarantini *et al*, 2008). iNOS demethylation is expected to increase expression and activity of the iNOS protein, which could in turn contribute to inflammation and oxidative stress generation, which are primary mechanisms linking inhalation of air pollutants to their acute health effects (Alexeeff *et al*, 2008; Baccarelli *et al*, 2007; Chahine *et al*, 2007). In the same study, long-term exposure to PM₁₀ was negatively associated with methylation in both Alu and LINE-1 (Tarantini *et al*, 2008). In a recent investigation, we showed that exposure to black carbon (BC), a marker of particles from vehicular traffic, was also associated with decreased DNA methylation in LINE-1, measured in 1,097 blood DNA samples from the Normative Aging Study (NAS), an investigation of elderly men in the Boston area. As repetitive element hypomethylation is believed to occur in patients with cancer (Ehrlich, 2002) and cardiovascular disease (Castro *et al*, 2003), such changes may reproduce epigenetic processes related to disease development and represent mechanisms by which particulate air pollution affects human health (Baccarelli *et al*, 2008c). Several in-vitro studies have established an association between DNA methylation and environmental metals, which are components of PM, including nickel, cadmium, lead, and particularly arsenic (Bleich *et al*, 2006; Dolinoy *et al*, 2007b; McVeigh *et al*, 2001; Wright and Baccarelli, 2007).

In addition, in an animal study, Yauk *et al*. showed that sperm DNA of mice exposed to steel plant air was hypermethylated compared to control animals and this change persisted following removal from the environmental exposure (Yauk *et al*, 2008). This finding calls for further research to determine whether air pollutants produce DNA methylation changes that are transmitted transgenerationally.

In our laboratory, we also investigated whether DNA methylation changes are induced by low-benzene exposure in peripheral blood DNA of gasoline station attendants and traffic police officers. High-level exposure to benzene has been associated with increased risk of acute myelogenous leukemia (AML) (Snyder, 2002). In our study, airborne benzene exposure was associated with a significant reduction in LINE-1 and Alu methylation. Airborne benzene was also associated with hypermethylation in p15 and hypomethylation of the MAGE-1 cancer-antigen gene (Bollati *et al*, 2007). These findings show that low-level benzene exposure may induce altered DNA methylation reproducing the aberrant epigenetic patterns found in malignant cells, which in most reports have been found to exhibit repetitive element hypomethylation as well as either hyper- or hypomethylation of specific genes, depending on the gene function. Also, benzene-associated demethylation of repetitive elements may help explain the epidemiological data linking benzene exposure with increases risk of multiple myeloma (Costantini *et al*, 2008; Kirkeleit *et al*, 2008), which also exhibits reduced methylation in Alu e LINE-1 repetitive elements (Bollati *et al*, 2007).

Other exposures that are associated with increased risk of hematopoietic malignancies, such as persistent organic pollutants (POPs), have been associated with changes in repetitive element DNA methylation. Rusiecki et al. (Rusiecki *et al*, 2008) evaluated the relationship between plasma POP concentrations and blood global DNA methylation, estimated in Alu repeated elements, in 70 Greenlandic Inuit, a population presenting some of the highest reported levels of POPs worldwide. In this work, a significant inverse linear relationships was found for DDT, DDE, β -BHC, oxychlordane, α -chlordane, mirex, several PCBs, and sum of all POPs (Rusiecki *et al*, 2008). Because most of the exposures investigated increase oxidative stress in human tissues, it is possible that production of reactive oxygen species represents a unifying process to account for most of these findings across different chemicals (Baccarelli and Bollati, 2009; Wright and Baccarelli, 2007). Oxidative DNA damage can interfere with the ability of methyltransferases to interact with DNA (Valinluck *et al*, 2004), thus resulting in a generalized altered methylation of cytosine residues at CpG sites (Turker and Bestor, 1997).

Final Remarks

In the last few years, several investigations have examined the relation between exposure to environmental chemicals and epigenetics, and identified several toxicants that modify epigenetic marks. Most of the studies conducted so far have been focused on DNA methylation, whereas only a few recent investigations have studied the effects of environmental chemicals on histone modifications (Baccarelli and Bollati, 2009). In animal models, environmental effects that might involve epigenetic mechanisms have been shown to be transmitted trans-generationally. Whether transgenerational epigenetic effects of environmental exposures are present in humans, or whether epigenetic inheritance exists at all in humans, remains to be elucidated.

Nonetheless, growing evidence indicates that epigenetics holds substantial potential for developing biological markers to predict which exposures would put exposed subjects at risk and which individuals will be more susceptible to develop disease. In human studies, this will require the use of laboratory methods with enhanced precision, sensitivity, and coverage, so that epigenetic changes can be detected as early as possible and well ahead of disease diagnosis. For several exposures, it has been proven that chemicals can alter epigenetic marks and that the same or similar epigenetic alterations can be found in patients with the disease of concern and/or in diseased tissues. Future prospective investigations are needed to determine whether exposed subjects develop epigenetic alterations over time and, in turn, whether such alterations increase the risk of disease.

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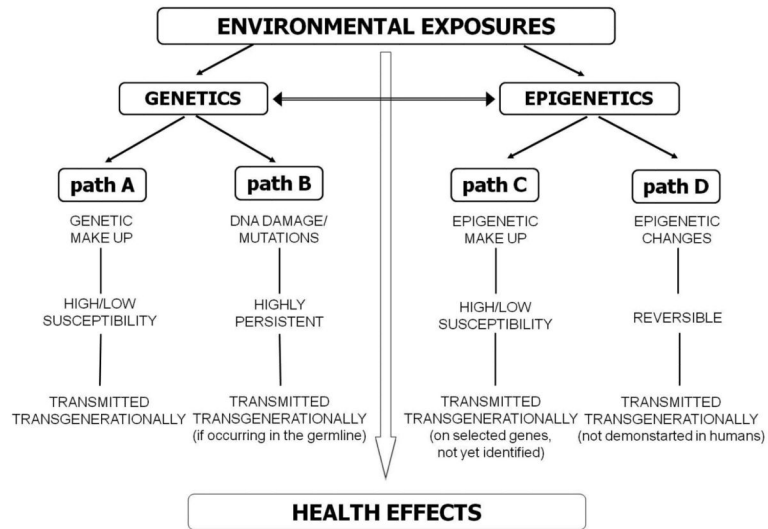


Figure 1. Gene—environment vs epigene—environment interplay: a model of possible genetic and epigenetic paths linking environmental exposures to health effects.