

## Meeting Report

# Environmental Epigenomics, Imprinting and Disease Susceptibility

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epigenomic targets, environmental exposures, fetal basis of disease, imprinting, susceptibility

## NOTE

The meeting website is: <http://www.geneimprint.com/meetings/2005durham/>.

## INTRODUCTION

On Tuesday, November 2, 2005 over 450 scientists representing 14 nations converged on the Washington Duke Inn, Durham, NC, USA to discuss, learn and exchange information on how environmental influences can exert impacts on health not only on the individual that has been exposed but also for up to four subsequent generations in some human and animal models tested. The meeting entitled "Environmental Epigenomics, Imprinting and Disease Susceptibility" was sponsored by the National Institute of Environmental Health Sciences (NIEHS) and the Duke Comprehensive Cancer Center. The meeting featured presentations from many of the leading authorities/experts in epigenomics in the world and approximately 70 poster presentations, of which twelve were selected for oral presentation. The meeting was organized into nine scientific sessions spread over two and a half days that addressed the fetal basis of disease, epigenetics and gene regulation, epigenetics and cancer, therapeutic and reproductive cloning, stem cell differentiation, epigenetics and chronic diseases and epigenetics and neurodevelopment. The opening session introduced the meeting co-organizers, Randy Jirtle of Duke University Medical Center and Frederick Tyson of NIEHS, to conference participants and included greetings from Christopher Willett, Chair of Duke Radiation Oncology Department, and William Schlessinger, Dean of the Nicolas School of the Environment and Earth Sciences. David Schwartz, Director of the NIEHS, set the tone for the conference with an overview lecture that identified research priorities of the NIEHS and pointed out the intersections between the environmental genomics component of NIEHS priorities and the environmental epigenomics. He noted that NIEHS research priorities will emphasize and coordinate efforts aimed at the study of complex human diseases. The environmental genomics infrastructural resources developed by NIEHS including over 500 re-sequenced environmentally responsive genes, over 50 humanized mouse strains, and progress towards establishing gene expression standards are available for utilization in the integration of epigenomic studies and the analysis of complex human diseases. Just as epigenomics is becoming increasingly more important in Schwartz's own asthma research, this conference identified additional opportunities for the integration of environmental epigenomics and complex human disease.

## FETAL BASIS OF DISEASE

The first session of the meeting focused on the fetal basis of adult disease paradigm. This paradigm states that the in utero environment is sensitive to perturbations by nutrition or environmental stressors. The effect of altered nutrition, as measured by low birth weight, or exposures to environmental stressors, as measured by altered gene expression, result in an animal or human that is more susceptible to disease/dysfunction later in life. Indeed, some of the effects of in utero exposures (nutritional or environmental stressor) can be passed on to future generations via what has been called altered programming. It is now apparent that altered programming refers to epigenetic changes and this session focused on how epigenetic changes in utero could lead to altered phenotypes with increased susceptibility to disease later in life. The first talk, Marcus Pembrey (Institute of Child Health, University College, London), focused on human studies of nutrition during the slow growth period of 7–12 years, just prior to puberty. He referenced a study that showed that paternal grandfather's good food supply during this slow growth period was associated with greater diabetes mortality in his grandsons but not the granddaughters. Similarly the paternal grandmother's food supply during this slow growth period just affects the mortality rate of her granddaughters and not her grandsons. He then showed an Avon Longitudinal Study of Parents and Children that studied cigarette smoking in

fathers and showed that paternal smoking can create a predisposition in children of health problems including obesity. The biggest effect occurred when the father started smoking between ages of 7–12 (prepuberty). This effect is thought to occur due to epigenetic changes that are passed via the sperm. Thus he showed that sperm are sensitive to epigenetic marks during the prepubertal period. These studies on a transgenerational response to smoking and nutrition that are sex specific and dependent on specific exposure periods, adds a new dimension to gene environment interactions in health and disease.

Robert Waterland (Baylor College of Medicine) continued the focus on transgenerational effects of nutrition, but with a focus on an animal model, the viable yellow agouti mouse. He showed that these genetically identical mice could be born with different coat colors that represented differential activity of the agouti gene. The coat colors could be modified by dietary manipulation of folate, B12, betaine and choline: all agents that impinge on the one carbon pathway. Increasing the amounts of these agents in the diet during pregnancy resulted in animals born with altered coat color that could be shown to coincide with increased methylation of the agouti gene locus. Thus supplementing the mother's diet with methyl donors resulted in permanent increases in methylation of the agouti gene which reduced its expression. The offspring of the supplemented animals were obese, indicating that the agouti gene was expressed in many tissues not just hair.

The final talk, John McLachlan (Tulane University), focused on the role of environmental agents, those with estrogenic activity and how in utero exposure to these agents can alter gene expression via epigenetics to increase susceptibility to disease and dysfunction later in life. He described experiments where neonatal female mice were treated for five days with estrogen or diethylstilbestrol (DES), a chemical with estrogenic activity given to millions of women in the 1950–70s to prevent miscarriage. He showed that neonatal exposure to estradiol or DES resulted in persistent phenotypic changes in the uterus and ovaries. He then showed that estrogens upregulated some genes during development in these tissues and that some are downregulated and that these changes persisted long after estrogen stimulation. This is different from the adult where estrogen is usually a reversible signal. The upregulated genes were shown to have altered methylation. Thus environmental estrogens have the potential via altering epigenetic marks to control gene expression. This expression then alters susceptibility to disease later in life, as some of these animals develop uterine cancers. He proposed that there might be a developmental estrogenization syndrome that would be due to altered gene expression as a result of altered epigenetic marks (methylation) or imprinting by developmental exposure to agents with estrogenic activity. Finally, he showed that plants use estrogens in the nitrogen fixation process. Thus not only animals but plant health might be altered by exposure to estrogenic influences at the wrong time or in abnormal concentrations.

## THERAPEUTIC AND REPRODUCTIVE CLONING

A session on therapeutic and reproductive cloning suggested that nonhuman primate embryonic stem cells and animals cloned by somatic cell nuclear transfer can be extremely useful to study the epigenomic regulations and mechanisms of genomic imprinting. Gerald Schatten (University of Pittsburgh) discussed the promise of human therapeutic cloning, in which embryonic stem cells (hESCs) are derived from somatic cells taken from the patient via nuclear



Figure 1. The painting on the cover of the program by the Portland, Oregon artist, Collin Murphy ([site-for-art.com](http://site-for-art.com)), is entitled "Origins." It vividly shows that identical twins can vary in their susceptibility to adult diseases because even though their genomes are the same, the epigenomes often vary markedly due to different environmental exposures during pregnancy.

transfer blastocysts, in allowing patient-specific, immune-matched cells to be used for stem cell transplantations. He explained that with the introduction of differentiated somatic nucleus in nuclear transfer, the reprogramming machinery of the cytoplasm must reorganize the new nucleus into a functional, pluripotent genome capable of carrying out proper differentiation of placental and fetal cell lineages. Dr. Schatten suggested that nonhuman primate embryonic stem cells and nuclear transfer embryonic stem cells can be useful for studying differentiation stability and other issues relevant to stem cell transplantation applications. Jorge Piedrahita (North Carolina State University) discussed some of the genome-wide and locus-specific methylation patterns in cloned animals that have identified abnormal epigenetic reprogramming at some loci. Both speakers suggested that epigenetic dysregulation of selected imprinted genes can occur during cloning by somatic cell nuclear transfer. He presented data suggesting that cloned animals can have phenotypic and developmental abnormalities due to epigenetic dysregulation of selected imprinted genes, and comparative genomics approaches can be useful to investigate the mechanisms of this dysregulation. Dr. Piedrahita described the observation that the severity of dysregulation of these imprinted genes appears to be greater in placental tissues than in fetal tissues. He proposed the hypothesis that the placenta is more susceptible to imprinting because it is the gate or interface between mother and

child. Furthermore, he suggested that this phenomenon may have implications for Intrauterine Growth Restriction and other placental-related phenotypes in humans. He is presently studying the role of imprinted genes in both normal and intrauterine growth restricted placentas. Dr. Piedrahita presented results that suggest that there are two types of imprinted genes: ones that can be very tightly regulated and others where the silent allele can be expressed to varying degrees.

## STEM CELL DIFFERENTIATION

This session featured talks that discussed epigenetic regulation on differentiation, stem cell fate determination, and potential therapeutic opportunities. The presenters in this session used different model systems to poignantly demonstrate that stem cell fate and phenotypes are strongly influenced by epigenetic mechanisms. Joanne Kurtzberg (Duke University) opened the session with a discussion of umbilical cord blood transplantation and the promise it brings for treatment of inborn errors of metabolism as well the transdifferentiation of cord stem blood cells into other cell types, e.g., oligodendrocytes and cardiac myocytes. She demonstrated several instances where cord stem blood cells have been used in patients for treatment of pathologic conditions, e.g., lysosomal storage diseases, congenital immunodeficiency syndromes, hemoglobinopathies, and bone marrow failure syndromes. Kurtzberg indicated that more than 6,000 transplantations of cord stem blood cells have been performed in the world. Cord stem blood cells allow for transplantation across HLA barriers for patients unable to find matched adult donors. This important clinical resource described by Dr. Kurtzberg was a demonstration of clinical utility for multi-potent cord blood stem cells that do not have terminal epigenomic differentiation status.

The next presentation of the session focused on the role of epigenetic regulation in determination of cell fate. Paul Wade (NIEHS), using human breast cancer cell lines to model Epithelial to Mesenchymal Transitions (EMT) in metastatic cancer, demonstrated cell differentiation fates are defined by chromatin modifications. This activity is regulated by the balance between two different signaling systems. Moreover, his presentation proposed an “epigenetic code” governs enzymes of nuclear metabolism; that a range of cellular stimuli can exert impact on gene expression through chromatin modification; and the differentiation of cells and phenotypic fates are reliant on epigenetic mechanisms.

Haifin Lin, (Duke University) identified the *Drosophila* PIWI gene as an important component in the transcriptional silencing pathway by epigenetic regulation and post transcriptional gene silencing. Lin presented data suggesting PIWI may be involved in regulating the epigenetic status of its genomic targets. The PIWI protein is associated with small non-coding RNAs transcribed from Telomeric Associated Sequences (TAS) and is responsible for maintaining the euchromatic status of TAS. He noted that this was opposite of PIWI function in maintaining the heterochromatic status of centromeric regions.

## EPIGENETIC GENE REGULATION I

This session started with a discussion of the history of epigenetics and how the organism uses the current environment to predict the future environment via epigenetic marks by David Haig (Harvard). He discussed the fact that environmental effects can result in DNA damage, repair, or predictive responses. Moreover, he noted that these predictions may cause problems leading to disease and dysfunction if the current environment fails to predict the future environment.

This is exactly what happens in the fetal basis of adult disease paradigm when human infants are exposed to malnutrition during development and then abundance of food later in life results in obesity, cardiovascular and other problems because the current environment was not predicted by the in utero environment.

The next two talks focused on imprinting in embryo culture (Marisa Bartolomei, University of Pennsylvania) and sex chromosome inactivation (Jeannie Lee, Harvard). Dr. Bartolomei pointed out that genomic imprinting is defined as unequal expression of the maternal and paternal alleles of a gene. This imprinting or epigenetic mark is added in the gametes and DNA methylation is the candidate for the mark. Her work focuses on genomic imprints in pre-implantation embryos in culture. Culture of embryos in vitro results in loss of some imprints indicating that the mechanisms that maintain imprinting were disrupted by the culture conditions. Indeed she showed that the perturbations in imprinting persisted long after the embryos were removed from culture. These in vitro data are supported by numerous publications that show that Assisted Reproductive Technology (ART) children have imprinting disorders. These results provide a strong cautionary note for the culture of human embryos since the effect of suboptimal culture on epigenetic information in humans is not known.

The inactivation of the X chromosome was the focus of the talk by Dr. Lee. She described the phenomenon of sex chromosome inactivation as one of the most fascinating problems in biology. In this case, one of the X-chromosomes in females is silenced by epigenetic marking for dosage compensation. This phenomenon is similar to autosomal imprinting where one allele is silenced in a sex dependent manner. She showed that some of the similarities are cis-acting control centers, long-distance regulation, differential DNA methylation, differential use of chromatin marking, and the presence of non-coding and antisense RNAs. She then asked the question: Does X-inactivation and genomic imprinting have a common evolutionary origin? She proposed that X chromosome inactivation and imprinting might have evolved from an ancient genome-defense mechanism that silences unpaired DNA. She also noted that X chromosome inactivation in mammals evolved from an ancient silencing mechanism that was first described in *N. crassa* and that it is conserved throughout evolution.

## EPIGENETIC GENE REGULATION II

The second session on epigenetic gene regulation focused on chromatin remodeling, histone modifications, and DNA methylation. These processes are epigenetically modified, dynamic mechanisms which allow distinct transcriptional control and may be important targets for many human disease therapies. Trevor Archer (NIEHS) has utilized the Mouse Mammary Tumor virus (MMTV) regulatory sequences as a model for understanding the role of chromatin and epigenetics in gene regulation. He has elucidated the role of chromatin remodeling and architecture in restricting the binding of ubiquitous transcription factors in steroid hormone dependent systems. Recently, he has attributed specific functions for subunits of the remodeling complex and unique intramolecular functions to the BRG1 ATPase component. He illustrated that the ability of chromatin remodeling proteins to disrupt the promoter architecture is itself regulated by epigenetic modification of the core histones at the promoter as well and that this is a distinct and dynamic transcriptional control mechanism.

Douglas Ruden (University of Alabama) described the model of epigenetic canalization which allows stability of epigenetically assimilated traits and phenotypes that are maintained for future generations. He discussed the example of Hsp90 in *Drosophila* where disturbances in chromatin-inheritance genes by stress or other factors can reveal previously masked morphological phenotypes in the following generations and a metastable allele can be rapidly selected. Ruden proposed that mechanisms of trans-generational epigenetic effects of DES and cancer in general may have similarities to the epigenetic phenomena observed in Hsp90 in *Drosophila*. He discussed the hypothesis that epigenetic canalization may occur in cancer because inheritance of tumor-promoting metastable epialleles are selected in precancer cells as they progress to the malignant state. Dr. Ruden described studies that have now shown that diet and stress can lead to hypomethylated CpG islands and may accelerate the epigenetic modifications that can produce metastable epialleles. Dr. Ruden suggested that 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 disease etiology and therapeutic intervention.

Andrew Hoffman (Stanford University) described the mechanism of imprinting loss at the adjacent insulin-like growth factor 2 (IGF2) and H19 genes which often occurs in tumors such as osteosarcomas. He showed that this loss may involve an incomplete gain or loss of methylation at a CTCF-binding site during tumorigenesis. Aberrant IGF2/H19 imprinting errors in *in vitro* fertilization (IVF) and assisted reproduction technologies may involve altered DNA methylation and histone methylation at an epigenetic switch of the IGF2/H19 genomic region, which may lead to an increased frequency in Angelman and Beckwith-Weidemann syndrome. He described recent insights into the IGF2-H19 region suggesting that reciprocal access to a single set of enhancers is regulated by parent-specific chromatin looping. This epigenetic reprogramming of IGF2 loss of imprinting can be corrected in tumor cells with nuclear transfer of normally imprinted IGF2. Dr. Hoffman concluded by proposing that interference with epigenetic reprogramming and canalization in cancer cells can be an effective strategy to target a variety of cancers and other epigenetically-influenced diseases.

## EPIGENETICS AND CANCER

This session offered some classical perspectives illustrating that disease and cancer can be attributed to sequence variation within coding regions of genes. In addition, some presentations provided evidence that alterations and modifications within non-coding regions can also regulate gene expression patterns.

Globally, DNA is often hypomethylated and thus linked to genomic instability and non-disjunction. Conversely, hypermethylation of genes is associated with aberrant gene silencing. Indeed, Manel Esteller, from the National Cancer Center (Spain) provided evidence that DNA hypomethylation induced by zebularine, a DNA methylation inhibitor occurred in association with depletion of extractable DNA methyltransferase 1 protein in mice. Thus, he proposed this inhibitor as a potential therapeutic agent with anti-tumorigenic activity. Moreover, evidence was provided to show that histone modification in concert with DNA methylation could contribute to epigenetic instability. Specifically, he showed that histone H4, important in DNA repair, undergoes both demethylation and deacetylation in mouse and human tumor cells. These results thus implicate a role for histone acetyl transferases, histone deacetylases, and histone methyl transferases in oncogenesis.

Along these same lines, Victor Lobanenko from the NIH showed that the CTCF paralog, Brother of the Regulator of Imprinted Sites (BORIS), acts like 5-azaC in inducing cancer gene activation (hypomethylation). BORIS is a mammalian 11 Zn-finger protein that mediates site specific interactions with 50-bp targets. It is believed that these targets regulate *in vivo* occupancy of such sites and may yield structurally and functionally distinct CTCF/DNA complexes involved in various aspects of gene regulation, including epigenetic control of gene imprinting and X chromosome inactivation. The latter functions are mediated by meCpG-sensitive 11ZF binding. Because CTCF is normally present in all somatic cells, whereas BORIS is active only in CTCF- and 5-methylcytosine-deficient adult male germ cells, Dr. Lobanenko believes that switching DNA occupancy from CTCF to BORIS may regulate site specificity and timing of epigenetic reprogramming.

The last talk of this session was by Jean-Pierre Issa (MD Anderson Cancer Institute), who provided evidence for environmental influences on epigenetic changes and how age-related silencing is commonly seen in various tissues and ultimately disease predisposition. The major question, however, was trying to discern when in cancer progression does aberrant methylation occur. He provided examples of DNA modification and aging such as ER- $\beta$  hypermethylation in vascular tissue. He also showed that epigenetic silencing is common in many tissues and related to chronic disease. Also of note there was an interesting model implicating lifestyle and the environment as a causal of epigenetic mosaicism as demonstrated with O(6)-methylguanine DNA methyltransferase hypermethylation and colon cancer.

## CHRONIC DISEASES

In mammals, changes in DNA methylation patterns are most dramatic during early development as well as in cancer. These events can be affected by environmental factors that can alter these patterns and levels. Indeed, precise patterns of methylation are needed for normal early development, genomic imprinting, and X-inactivation. The first talk in this session examined how higher order chromatin structure is dependent upon sequence and therefore regulates methylation and issues just described. Certainly it is known that imprinted genes and their control elements are clustered and subject to methylation. Adele Murrell (Cambridge University) provided some insight into how these elements are regulated. She showed that DNA methylation in the IGF2-H19 region of mouse can be altered if under the control of differentially methylated regions (DMRs) after implantation. These results showed that DMRs are actively coordinated in a stepwise fashion. Indeed, H19 DMR was required in hypomethylation of IGF2 DMRs 1 and 2, and concomitantly IGF2 DMR1 afforded DMR2 protection from methylation. The highlight from her study is the idea of regional coordinated protection from methylation happening mainly in embryonic development.

Bruce Richardson (University of Michigan) examined the role of gene-environment interaction and disease etiology. Specifically, he showed that the DNMT1 inhibiting drug procainamide elicits idiopathic lupus. Mechanistically this effect is thought to occur through hypomethylation as levels of methyltransferase are reduced in lupus T cells. Moreover, this effect is thought to alter gene expression as a result of regulatory regions being demethylated. This in turn is posed as a reason for T-cell auto-reactivity and ultimately macrophage killing. Other environmental agents are now being examined including those that inhibit the ERK pathway, which serves in cellular proliferation, differentiation, and survival, and cancer under inappropriate activation.

The last speaker, Donata Vercelli (University of Arizona), provided further evidence for the notable increase in autoimmune disease and asthma through the potential involvement of environmental factors and the allergic response. Specifically, studies were presented that examined IL-13 locus, a cytokine that plays a critical role in TH2-mediated inflammation, as a target for DNase I hypersensitivity sites and CpG methylation. She showed that chromatin structure undergoes drastic remodeling during differentiation in CD4<sup>+</sup> T cells. However this effect was not exclusive as TH1 cells absent of IL-13 reveal a similar pattern. This lead her to the idea that IL13 is actually resistant to condensed chromatin structure during early development as neonatal T cells carry out high expression at this locus. It is not until later in life that this regulation is altered and expression of IL13 leads to an allergic response, which at this juncture could be influenced by environmental factors.

## EPIGENETICS AND NEURODEVELOPMENT

This session featured presentations with compelling evidence for the profound effects of epigenetic programming on cognitive and behavioral development in humans and animal models. David Skuse (University College London) discussed X-linked genes and mental functioning. His presentation provided an overview of epigenetic mechanisms by which X linked genes can affect cognitive functioning and provided evidence for X-linked polymorphisms that affect emotion recognition. His research implies there is an X-linked QTL at Xp11.4 that influences social cognitive circuitry in Turner's Syndrome females (XO), autistic patients, and perhaps the overall male population. Moshe Szyf (McGill University) discussed epigenomic programming by maternal behavior with neonatal pups. He showed programming of glucocorticoid receptor (GR) activity in the hippocampus of neonates persists late into adulthood. This work is the first to demonstrate that maternal behavior evokes specific signaling pathways in the hippocampus of offspring that targets chromatin modification and demethylase activity in the GR exon 1 promoter. Moreover, his work also demonstrates that methylation patterns established by maternal behavior are long lasting but are potentially reversible by behavioral, physiological, and or pharmacological signals.

The final presentation of this session was made by Randy Jirtle (Duke University). He provided an overview of his work on the M6P/IGFR2 tumor suppressor gene demonstrating that M6P/IGFR2 imprinting is both species and tissue specific. His work provides strong evidence that haploinsufficiency of M6P/IGFR2 in the brain of male mice impairs cognitive ability as measured by early choice accuracy in the radial arm maze test. To address the question of whether the M6P/IGFR2 is an IQ gene in humans, he has identified a polymorphism in humans that is strongly associated with a reduction in IQ in males.

This session highlighted possibilities that are on the horizon for potential landmark discoveries on epigenomic mechanisms and pathways that influence neurodevelopmental outcomes, e.g., Alzheimer's, autism, cognitive deficiencies and provide hope for behavioral, physiological and or pharmacological interventions that be able to alter the equilibrium between methylated and demethylated genes.

An evening session that included a reception, poster viewing, and banquet for conference participants was highlighted by an informative discussion by Emma Whitelaw (University of Sydney) on metastable epialleles. Monozygotic twins can be very different phenotypically because of differential patterns of epigenetic gene silencing. Dr. Whitelaw's presentation highlighted her work with the agouti and

axin loci in inbred mice and bi-sulfite sequencing of human monozygotic twins to identify genomic sequences that are differentially methylated in different individuals. She and her colleagues have identified a number of sequences that have expressed differential epigenetic control and therefore differential expression in different individuals. These gene loci that can have epigenetically controlled differential expression in different individuals should be known as metastable epialleles.

The final session started with a meeting overview by Andrew Feinberg (Johns Hopkins University School of Medicine). He started this daunting task by reminding the audience that while DNA is the alphabet of life, epigenetics is the grammar of life. It is the adjectives and adverbs that give meaning (DNA methylation), the sentence structure (DNA coiled around histones which are covalently modified), and the chapter organization (information in the organization of loops). He then pointed out that the really big question is what are the roles of genetics and epigenetics and how are they inter-related and how do they contribute to human disease. Could disease really be caused by grammatical errors in the epigenome? To really assess this question we really need a human epigenome project to identify all of the biochemical changes and relationships among genes that provide function to the DNA code, which will allow understanding of normal development and aging, abnormal gene control in cancer and other common diseases and the role of the environment in human health. He described two projects that would partly address these issues: the Human Cancer Genome Project and the ENCODE (ENCyclopedia of DNA Elements) project that would both include analysis of epigenetic marks. The problem of course, as he noted, is how can one analyze so many epigenomes? He proposed a comprehensive analysis of a small number of reference epigenomes, directed analysis of larger numbers of epigenomes, and technology development to obtain a reference set of tools and reagents. He also described a specific example of a common epigenetic variant associated with a common disease: loss of imprinting of the insulin-like growth factor II gene in various cancers.

## FINAL SESSION AND RECOMMENDATIONS

Terry and Joe Graedon, co-hosts of the People's Pharmacy PBS radio show, then led the general discussion. The following points were noted during an extensive and energetic discussion:

- Epidemiology studies are needed using case control studies followed over time or studies that would plug into ongoing studies and would add epigenetic endpoints. One example would be to use the DES cohort and reexamine them for a correlation of disease endpoints with epigenetic changes in gene expression.
- Monozygotic twin studies are needed to examine the interaction of environmental exposures and epigenetic changes over time.
- More transgenerational studies are needed in animal models. These should then provide information that could lead to an examination of transgenerational effects in humans that would be linked to epigenetic changes.
- A continued focus on the fetal or developmental basis of disease paradigm is needed with a focus on both nutrition and environmental exposures and their interactions as well as epigenetic modification as the mechanism for the increased susceptibility to disease later in life from the developmental exposures. In this regard we need to validate mouse models using outbred animals to mimic the human situation.

- An epigenome project is needed and it should be done with International coordination.
- More data are needed on the best way to biobank tissues and serum for future epigenetic analyses. We can now biobank tissues and examine them for methylation changes but don't know for sure how to preserve chromatin structure or histone modifications.
- We need to know the epigenome of germ cells and oocytes.
- We need to know the epigenome of stem cells. A stem cell should be defined epigenetically.
- New technologies and informatics are needed for the analysis of epigenomic data.
- Finally it was noted that we are in the infancy of opportunity in the field of environmental epigenetics.