 EH298

Environmental Epigenetics

Harvard School of Public Health – Fall 2015

Instructor

Andrea Baccarelli
Mark and Catherine Winkler Associate Professor of Environmental Epigenetics
Harvard School of Public Health
Departments of Environmental Health & Epidemiology
SPH-1, Rm-G5;
Phone: (617) 432-2095
Email: abaccare@hsph.harvard.edu

Teaching Assistant

Jia Zhong
Doctoral student
Environmental/Occupational Molecular Epidemiology
Harvard School of Public Health
SPH-1, Rm-G11
Phone: (617) 432-0666
Email: jiakhong@mail.harvard.edu
Course Description

Epigenetics is a fast growing field, with increasing applications in epidemiology and environmental health studies. Epigenetics focuses on the alterations in chromatin structure that can stably and/or heritably influence gene expression. Epigenetic changes can be as profound as those exerted by mutations, but, unlike mutations, are reversible and responsive to environmental influences. The course will focus on biological mechanisms, laboratory methods, and research applications of DNA methylation and histone modifications. Other emerging epigenetic mechanisms will be introduced. Ongoing experimental and epidemiology studies (cohort, case-control, cross-sectional and repeated measurement studies) will be presented to introduce the students to the epigenetic effects in prenatal/early and adult life risk factors, including toxic exposures (air pollution, metals, smoking, BPA, benzene, PCBs, and persistent organic pollutants), smoking, diet and nutrition, and psychosocial risk factors. The course will enable the students to evaluate and apply epigenetic methods in multiple areas, including cardiovascular and respiratory disease, aging, reproductive health, inflammation/immunity, cancer, and neuropsychological disorders.

Meeting Times

Lectures will be on Tuesday and Thursday, from Sep 3rd through Oct 22nd, 1:30 to 3:20 pm. Student presentations will be on Tuesday Oct 20th and Thursday Oct 22nd, 1:30 to 3:20 pm.

Course Materials

Assigned readings will be primary literature from recently published articles, as indicated below for each of the lectures. Landmark historical articles are also indicated. Links to the PDF files of the papers (through the Harvard University System) can be opened by clicking on the citations on each of the lecture pages.

In-class reviews of recent original articles will be used to introduce the students to the study design, statistical, and laboratory methods used in epigenetics investigations, as indicated below. The students are required to read the articles in advance of the in-class reviews. The links to the PDF articles of the papers proposed for the in-class reviews are accessible by clicking on the citations. The students will have the choice to either print the PDF of the articles before the class or access the PDFs online using their laptops during the class.
Course Objectives

At the completion of the course, the student will be able to:

1. Describe the concepts of epigenetics, including DNA methylation, histone modifications, and other epigenetic mechanisms.
2. Evaluate the different laboratory methods for epigenetics, including MSP, Methylight (quantitative real-time PCR), Pyrosequencing, Sequenom MALDI-TOF, chromatography, and genome-scale technologies (microarrays and next-generation sequencing), and their use in epidemiology and environmental health research.
3. Learn and apply statistical methods for analysis of epigenetic data.
4. Evaluate and design epidemiology and environmental investigations that examine the effects of common risk factors on epigenetic states.
5. Evaluate and design epidemiology and environmental investigations that examine the correlation of epigenetic states with health outcomes related to cardiovascular and respiratory disease, aging, reproductive health, neurological and neuropsychological diseases, and cancer.

Outcome Measures

Class Attendance and Participation
Active learning through class participation and discussion are an important component of the course. The course will meet for eleven 2-hour sessions over seven weeks. Two additional sessions will be reserved for student presentations on the last week of classes. Students are required to attend and participate in all classes.

Presentations
Students are expected to give a short presentation during the sessions indicated in the class schedule (Lecture 14 and Lecture 15). For their presentations, students are encouraged to choose from the topics listed in the class schedule. Group presentations are encouraged. Presentation time (usually 5-10 min) will be allocated to each student based on the number of students in the class.

Homework Assignments
Students will be asked to prepare brief written assignments that will have the objective to review the topics discussed during the lectures and/or described in the homework readings. Please see next page for details on the homework schedule.

Grading Criteria
Student grades will be based on class participation (10%), homework assignments (50%), and class presentations (40%).
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<thead>
<tr>
<th>Week</th>
<th>Lecture</th>
<th>Date</th>
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<tr>
<td>1</td>
<td>No Class</td>
<td>Tue Sep 1st</td>
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<tr>
<td>1</td>
<td>1. -Introduction and General Concepts</td>
<td>Thurs Sep 3rd</td>
<td>1:30-3:20pm</td>
<td>A. Baccarelli</td>
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<td>-DNA Methylation</td>
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<td>2. -Histone Modifications</td>
<td>Tue Sep 8th</td>
<td>1:30-3:20pm</td>
<td>A. Baccarelli</td>
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<td>2</td>
<td>3. -Environmental Influences on the Epigenome</td>
<td>Thurs Sep 10th</td>
<td>1:30-3:20pm</td>
<td>A. Baccarelli</td>
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<td>3</td>
<td>4. -Technologies for DNA Methylation Analyses</td>
<td>Tue Sep 15th</td>
<td>1:30-3:20pm</td>
<td>A. Baccarelli</td>
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<td>5. -Tissue Specificity in Epigenetics</td>
<td>Thurs Sep 17th</td>
<td>1:30-3:20pm</td>
<td>A. Baccarelli</td>
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<td>-Introduction to statistical methods for cell type estimation</td>
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<td>-In-class review: Study Design in Epigenetics</td>
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<td>6. -Inherited Diseases of the Epigenetic Machinery</td>
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<td>1:30-3:20pm</td>
<td>A. Baccarelli</td>
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<td>4</td>
<td>7. -From GWAS to EWAS: Design and Conduction of Epigenome Wide</td>
<td>Thurs Sep 24th</td>
<td>1:30-3:20pm</td>
<td>A. Baccarelli</td>
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<td>Association Studies (EWAS)</td>
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<td>-In-class review: Interpretation of Epigenetic Data</td>
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<td>8. -Guest Lecture: Reproductive Epigenetics and Prenatal Influences</td>
<td>Tue Sep 29th</td>
<td>1:30-3:20pm</td>
<td>K. Michels</td>
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<td>H. Burris</td>
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<td>9. Lab Session: Statistical Analysis of EWAS data</td>
<td>Thurs Oct 1st</td>
<td>1:30-3:20pm</td>
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<td>10. -Application of Epigenetics on Aging and Chronic</td>
<td>Tue Oct 6th</td>
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<td>6</td>
<td>11. -Guest Lecture: Epigenetics of Obesity</td>
<td>Thurs Oct 8th</td>
<td>1:30-2:20pm</td>
<td>G. Agha</td>
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<td>-Application of Epigenetics on Aging and Chronic Diseases (2/2)</td>
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<td>12. -Environmental Mitochondriomics</td>
<td>Tue Oct 13th</td>
<td>1:30-3:20pm</td>
<td>A. Baccarelli</td>
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<td>13. -Introduction to Non-coding RNAs and New Topics in Epigenetics</td>
<td>Thurs Oct 15th</td>
<td>1:30-3:20pm</td>
<td>A. Baccarelli</td>
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<td>8</td>
<td>14. -Student Presentations, Session #1</td>
<td>Tue Oct 20th</td>
<td>1:30-3:20pm</td>
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<td>15. -Student Presentations, Session #2</td>
<td>Thurs Oct 22nd</td>
<td>1:30-3:20pm</td>
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Homework Schedule

**Week 1 - Thurs. Sep 3rd (Due before class, Thurs. Sep 10th)**
Written assignment 1 – Definitions and Epigenetic Inheritance

**Week 2 - Thurs. Sep 10th (Due before class, Thurs. Sep 17th)**
Readings for in-class review of epigenetic papers: Study Design in Epigenetics*
Written assignment 2 – Bisulfite Treatment and Primer Design
*Reading is due before next Thursday’s lecture

**Week 3 - Thurs. Sep 17th (Due before class, Thurs. Sep 24th)**
Readings for in-class review of epigenetic papers: Interpretation of Epigenetic Data*
Written assignment 3 – Tissue Collection, Tissue Specificity, and Lab Methods
*Reading is due before next Thursday’s lecture

**Week 4 - Thurs. Sep 24th (Due before class, Thurs. Oct 1st)**
Written assignment 4 – Warm-up for the Data Analysis

**Week 5 - Thurs. Oct 1st (Due before class, Thurs. Oct 8th)**
Written assignment 5 – Data Analysis

**Week 6 - Thurs. Oct 8th (Due before class, Thurs. Oct 15th)**
Written assignment 6 – Study design: Epigenome Wide Association Studies (EWAS)

**Week 7 - Thurs. Oct 15th**
Prepare concepts for student presentations: Sign up for student presentations

**Week 8 - Tue Oct 20th and Thurs. Oct 22nd**
Deliver student presentations

Office Hours

Jia Zhong – Time: Tuesday 12:30-1:30pm; Location: Building 1, 1306A (Office hour will be held in 1306B on Sep. 29th)
Dr. Andrea Baccarelli – By appointment (Please email Jia to schedule an appointment)
Laboratory of Environmental Epigenetics

Our laboratory of Environmental Epigenetics is located in rooms SPH1- G13. Students interested in visiting the lab or practical laboratory activities are encouraged to discuss their interests with the teaching staff.

General Reading Material

Michels, Karin B. Epigenetic Epidemiology. Springer 2012
Week 1

No Class (Tue, Sep 1st 1:30-3:20 pm)

Lecture 1 (Thurs, Sep 3rd 1:30-3:20 pm)

**Introduction and General Concepts; DNA Methylation**
- Introductions and Syllabus Review; Q & A.
- Epigenetics: From Phenomenon to Field
  - History
  - Definition
  - Recent Developments
- DNA Methylation: Definition and Mechanisms

**Reading Material**
- Michels K. Chapter 2 – The Human Epigenome, In Epigenetic Epidemiology, Michels K (Editor), Springer 2012; pag 5-20.

**Learning Objectives**

1. Differentiate between genetic and epigenetic codes
2. Analyze recent development and current interest in epigenetics
3. Describe the most well-known epigenetic mechanisms
4. Explain how epigenetics mechanisms regulate gene expression
5. Evaluate the nature of epigenetic variations and how these variations may cause disease susceptibility
Lecture 2 (Tue, Sep 8th 1:30-3:20 pm)

Histone Modifications
- Histone Modifications: Definition and Mechanisms
- Simple Methods for Histone Modification Analysis in Epidemiology Studies
- Case Study: Effects of Exposure to Metal-rich Air Particles on Histone H3-K4 Dimethylation and H3-K9 Acetylation among foundry workers.

Reading Material

Lecture 3 (Thurs, Sep 10th 1:30-3:20 pm)

Environmental Influences on the Epigenome
- Effects of Environmental Toxicants on the Epigenome
  - Potential mechanisms
  - Effects on global methylation content; effects on gene-specific methylation
- Review of Environmental Investigations
  - Investigations on air pollutants and toxic metals
  - Investigations on peroxisome proliferators (TCE, DCA, TCA)
  - Investigations on Endocrine-disrupting Chemicals and Reproductive Toxicants
- Case Study: Epigenetic effects of air pollution in the Milan Benzene Study

Reading Material:

Learning Objectives
1. Describe selected laboratory methods for histone modification analysis
2. Analyze how environmental exposures may produce epigenetic alterations
3. Describe the relevance of epigenetic changes in mediating the effects of environmental exposures
4. Analyze available evidence from in-vitro, experimental and human studies
5. Position the potential role of epigenetics in the mechanisms of action of air pollutants, metals, pesticides, PCBs & Dioxins and other pollutants
Week 3

Lecture 4 (Tue, Sep 15th 1:30-3:20 pm)

Technologies for DNA methylation Analyses

- Analysis of Global Methylation Content: HPLC, GC/MS
- Analysis of Gene-specific Methylation
  - MSP, Real-time PCR (Methylight), Pyrosequencing, Sequenom MALDI-TOF
- Methylation Microarrays and other Genome-wide Methods
  - Methylation microarrays, deep sequencing; comparison of available platforms

Reading Material
- Michels K. Chapter 4 – Laboratory Methods In Epigenetic Epidemiology. Epigenetic Epidemiology, Michels K (Editor), Springer 2012; pag 37-55
- Watababe, Y and Maekawa, A. Chapter 2 - Methods and Strategies to Determine Epigenetic Variation in Human Disease. In Tollesfsbol T (Editor), Epigenetics of Human Disease, Elsevier 2012; Pages 7-27

Lecture 5 (Thurs, Sep 17th 1:30-3:20 pm)

Tissue Specificity and Study Design in Epigenetics

- Use of non-target tissues in human investigations
  - Case Study: A Panel Study on Epigenetics, Markers of Oxidative Stress, and Lung Function among Children with Respiratory Disease Exposed to Industrial Air Pollution
  - Using AceView and NCBI Resources to identify tissue expression and gene functions
- Introduce statistical methods for cell type estimation
  - Using Houseman algorithm for estimating cell type proportions
  - Reference-free cell mixture adjustments for DNA methylation data analysis
- In-class Review of Selected Readings – Study Design in Epigenetics

Reading Material:
- Michels K. Chapter 3 - Consideration in the Design, Conduct and Interpretation of Studies, In Epigenetic Epidemiology, Michels K (Editor), Springer 2012; pag 27-36

Learning Objectives

1. Describe different methods for determining DNA methylation and their use in epidemiology and environmental health investigations
2. Review the use of epigenetic data and their interpretation
3. Identify challenges in the use of surrogate tissues in epidemiology
4. Learn statistical methods for cell type estimation
5. Analyze the advantages and pitfalls of different designs in the conduction of epidemiology studies
Inherited Disease of the Methylation Machinery

- Inherited Diseases of the Epigenetic Machinery
  - Rett Syndrome
  - ICF Syndrome
  - ATR-X Syndrome
  - Fragile X Syndrome

Reading Material

From GWAS to EWAS: Design and Conduction of Epigenome Wide Association Studies (EWAS)

- Conduction of GWAS
- Study design in EWAS -- Case study:
  - discovery only
  - discovery with internal replication – split design
  - discovery with external replication
  - cross validation
  - meta-analysis
- In-class Review of Selected Readings – Interpretation of Epigenetic Data
  - Lee et al. Quantitative promoter hypermethylation analysis of RASSF1A in lung cancer: Comparison with methylation-specific PCR technique and clinical significance
  - Joubert et al. 450K Epigenome-Wide Scan Identifies Differential DNA Methylation in Newborns Related to Maternal Smoking during Pregnancy

Reading Material

Learning Objectives

1. Describe the concept of loss of imprinting
2. Review the most commons syndromes associated with alterations at imprinted loci
3. Describe the rare inherited conditions associated with epigenetic states
4. Review the use of epigenetic data and their interpretation
5. Review EWAS study designs.
Week 5

Lecture 8 (Tue, Sep 29th 1:30-3:20 pm)

Reproductive Epigenetics and Prenatal Influences on the Epigenome

- **Guest lecture (1:30-2:20 pm)**
  Karin Michels, PhD, ScD
  Co-Director, Obstetrics and Gynecology Epidemiology Center, Brigham and Women's Hospital, Harvard Medical School.

- **Guest lecture (2:30-3:20 pm)**
  Heather Herson Burris, MD, MPH
  Neonatology, Beth Israel Deaconess Medical Center.

Reading Material:

Lecture 9 (Thurs, Oct 1st 1:30-3:20 pm)

Lab Session:
Data Analysis of Epigenome Wide Association Studies (EWAS)

This will be a hands-on session on 450K Illumina Methylation Data from the Veteran Administration Normative Aging Study.

A masked database from the study will be distributed. We will walk through a complete EWAS analysis of DNA methylation and mortality. By the end of the session, students will gain understanding of epigenome wide data, as well as of common tools and methods for EWAS analysis.

The session will be conducted in R.

Learning Objectives

1. Review opportunities and challenges for perinatal epigenetic studies
2. Describe the epigenetic findings in animal models of maternal care and stress
3. Review the use of epigenetic data and their interpretation
4. Learn and apply statistical methods for EWAS analysis
Week 6

Lecture 10 (Tue, Oct 6th 1:30-3:20 pm)

Application of Epigenetics on Aging and Chronic Diseases (1/2)

- Epigenetics and Cancer
- Epigenetics and Cardiovascular Diseases
- Epigenetics and Neuropsychiatric Disorders

Reading Material

Lecture 11 (Thurs, Oct 8th 1:30-3:20 pm)

Application of Epigenetics on Aging and Chronic Diseases (2/2)

Guest lecture (1:30-2:20 pm) “Epigenetics and Obesity”
- The title is being reviewed by the guest lecturer and may be modified.

Golareh Agha, Ph.D.
Department of Environmental Health – Exposure, Epidemiology & Risk Program
Harvard School of Public Health, Boston.

Lecture (2:30-3:20 pm) “The Epigenetic Aging Clock”
- DNA methylation and cellular aging
- The twin model
- Epigenetics of aging phenotypes

Reading Material

Learning Objectives
1. Analyze the evidence for age-related changes in DNA methylation
2. Describe the twin model by Fraga et al. and discuss the role of the environment and age
3. Differentiate the roles of global and gene-specific hypo/hypermethylation in cancer etiology
4. Evaluate the evidence for the epigenetic effects of environmental carcinogens
5. Describe the role of epigenetic mechanisms in inflammatory processes and evaluate their potential impact on cardiovascular diseases
6. Describe the epigenetic patterns associated with cardiovascular outcomes
7. Review potential epigenetic alterations associated with neurological diseases
8. Identify opportunities for blood-based biomarkers in epigenetic neurological research
Week 7

Lecture 12 (Tue, Oct 13th 1:30-3:20 pm)

**Environmental Mitochondriomics**
- Environmental toxins and mitochondrial oxidation
- The role of mitochondrial DNA abundance in pollution-related health outcomes
- Mutations and deletions of the mitochondrial genome
- Mitochondrial DNA methylation: a potential biomarker for chronic diseases

**Reading Material**

Lecture 13 (Thurs, Oct 15th 1:30-3:20 pm)

**Introduction to non-coding RNAs and New Topics in Epigenetics**
- Noncoding (nc) RNAs: Classes and Functions
  - Biological Roles of ncRNAs
  - Laboratory Methods for ncRNAs Measurement
- Epigenetics and ncRNA
  - MicroRNA Control of Epigenetic Mechanisms
  - Epigenetic Control of MicroRNA Expression
- microRNA and the Environment
  - Environmental Influences on MicroRNA
  - Use of MicroRNA Markers in Environmental Health and Epidemiology
  - Case Study: Metal-rich Particulates and MicroRNAs among Foundry Workers
- New Topics in Epigenetics
  - 5-hydroxymethylcytosine and non CpG Methylation
  - Novel Findings for Epidemiology Studies: Stability of DNA markers

**Reading Material**

**Learning Objectives**
1. Review the roles of oxidation and mitochondria in environmental causation of disease.
2. Describe non-coding RNA function and biology.
3. Review novel emerging topics in epigenetics, including 5-hydroxymethyl cytosine, non CpG methylation and novel regulatory sequences.
Sessions 14 & 15
(Tue, Oct 20th & Thurs., Oct 22nd 1:30-3:20 pm)

**Students’ Presentations**
Students are expected to give a short presentation during the sessions indicated in the class schedule (Lecture 14 and Lecture 15). For their presentations, students are encouraged to choose from the topics listed in the class schedule. Presentation time (usually 5-10 min) will be allocated to each student based on the number of students in the class. Sign up for presentations by Thursday, Oct 8th.

For their presentations, students are encouraged to:
- Review direct or indirect evidence for the epigenetic effects of a specific environmental exposure or other risk factor of their interest.
- Review direct or indirect evidence for epigenetic alterations in a cellular pathway or in a disease of their interest.
- Prepare a proposal for a possible epigenetics investigation on a subject of their choice.

**Group presentations are encouraged.**