Environmental exposures and mechanisms of transgenerational epigenetic inheritance

To eat or not to eat: Is that the (only) question?

Raquel Chamorro-Garcia
Assistant Professor
Department of Microbiology and Environmental Toxicology
University of California, Santa Cruz
Share of adults that are obese, 1975

Obesity is defined as having a body-mass index (BMI) equal to or greater than 30. BMI is a person's weight in kilograms divided by his or her height in metres squared.
Share of adults that are obese, 2016

Obesity is defined as having a body-mass index (BMI) equal to or greater than 30. BMI is a person's weight in kilograms divided by his or her height in metres squared.

Source: WHO, Global Health Observatory
# Global impact of obesity

## Global Burden of Obesity

<table>
<thead>
<tr>
<th>Health</th>
<th>Economy</th>
<th>Social</th>
</tr>
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<tbody>
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<td>• Cardiovascular disease</td>
<td>• $2.0 trillion worldwide</td>
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- **Economy**
  - $2.0 trillion worldwide
  - Direct costs
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- **Social**
  - Developing countries cannot afford the costs
  - Stigma

- **Health**
  - Cardiovascular disease
  - Diabetes
  - Musculoskeletal disorders
  - Cancer
Contributing factors to obesity

- Positive energy balance (calories in vs. calories out)
  - Genetics
  - Stress
  - Gut microbiome
  - Infections
- Smoking
- Malnutrition

Childbirth and subsequent growth
Multigenerational studies in humans
Dutch famine

Preconception & in utero exposure to famine

Health Outcomes in offspring

F2 offspring from F1 women

Obesity, lipid profile, metabolic syndrome
Diabetes, glucose metabolism
Fertility
Cognition, psychiatric disorders
Adult mortality

Poor health
Multigenerational inheritance

One mutation

Genetics
Mendelian laws
Dominant vs recessive phenotype
Homozygous vs heterozygous vs WT

Epigenetics
Environmental perturbation

Obesity
Lipid profile
Metabolic syndrome
Diabetes
Glucose metabolism
Fertility
Cognition
Psychiatric disorders
Adult mortality

One phenotype

?
Epigenetics

Alterations that lead to a “stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence”

Positive energy balance (calories in vs. calories out)
  Genetics
  Stress
  Gut microbiome
  Infections

Contributing factors to obesity

EDCs?
Transgenerational epigenetic inheritance

Initial alteration

Transgenerational epigenetic inheritance

Final endpoint

Modes of action
Target tissues

Which parental line?
Epigenetic marks in germline

Mechanisms of propagation?
Epigenetic reprogramming?

EDCs
Tributyltin
Phthalates
Methoxychlor
Glyphosate
Dioxin
BPA

Diets
Caloric restriction
High-fat diet

Epigenetic Characterization
DNA methylation
Histone modifications
Noncoding RNAs

Exposure of F0
Pregnant Females

F1

F2

F3

F4

Metabolic disruption F4 animals

Chamorro-Garcia et al., 2013
Skinner et al., 2013
Tracey et al., 2013
Manikkam et al., 2013
Manikkam et al., 2014

Öst et al., 2014
Chamorro-Garcia et al., 2017
Camacho et al., 2018
Kubsad et al., 2019
Diaz-Castillo et al., 2019
Tributyltin
Biocide
Seafood
Metabolic disruptor

Exposure of F0 Pregnant Females

F1  F2  F3  F4  Metabolic disruption F4 animals

F1  F2  F3  F4

Tributyltin
Biocide
Seafood
Metabolic disruptor
Exposure of F0 Pregnant Females

Tributyltin
Biocide
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SD: Standard Diet
HFD: High Fat Diet

Chamorro-Garcia et al., Nat Comms, 2017

Exposure of F0 Pregnant Females

Metabolic disruption F4 animals

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Chamorro-Garcia et al., Nat Comms, 2017
Tributyltin

Exposure of F0 Pregnant Females

F1 F2 F3 F4

Metabolic disruption F4 animals

Tributyltin

Hypothesis I: TBT alters DNA methylation of promoters for metabolically-relevant genes

Rejected

Hypothesis II: TBT alters nuclear genome organization

F4 Adipose tissue

Alterations in methylome & transcriptome

Chamorro-Garcia et al., Nat Comms, 2017
Epigenetics

Alterations that lead to a “stably heritable phenotype resulting from changes in a chromosome without alterations in the DNA sequence”

Nuclear genome organization

(A) Eukaryotic cell

Nucleus

Euchromatin
Active genes, higher accessibility
High GC content

Heterochromatin
Transcriptionally inactive
Less accessible
High AT content

(B) Chromosome compartments

Active (A)
Inactive (B)

Compartment A
Euchromatin
Active genes
Higher accessibility
High GC content

Compartment B
Heterochromatin
Transcriptionally inactive
Less accessible
High AT content

Immunofluorescence

Rods
Non-rod neurons

Euchromatin
Heterochromatin

Hi-C

Schematic of compartment signal by Hi-C

Hildebrand & Dekker, 2020 – Trends Biochem Sci
Falk et al., 2019 - Nature
Tributyltin

Hypothesis I: *TBT alters DNA methylation of promoters for metabolically-relevant genes*

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Tributyltin

Isochores

- Large regions of DNA (>300 Kb) with highly homogenous base composition (GC- vs. AT-content)
- Reflect multiple levels of organization (TADs, eu-/heterochromatin, compartment A/B)
- Are invariable across tissues, generations and sexes
- Analysis of genomic traits with regards isochores before and after randomly rearranging datasets 10,000 times

Adipose tissue

Alterations in methylome & transcriptome

How?

F4

Exposure of F0 Pregnant Females

F1

F2

F3

F4

Metabolic disruption F4 animals
Heterochromatin-euchromatin organization disruption
TBT vs Control

Significantly higher than expected by chance in heterochromatin

Significantly lower than expected by chance in euchromatin

Not significantly different than expected by chance

isochores
AT-rich -> GC-rich
heterochromatin -> euchromatin
Exposure of F0 Pregnant Females

F1 → F2 → F3 → F4

Metabolic disruption F4 animals

Tributyltin

Molecular mechanism?

Fat tissue
Liver
MSCs

Males & Females

Transcriptome
& Methylome

Alterations in methylome & transcriptome

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Chamorro-Garcia et al., Nat Comms, 2017
Diaz-Castillo et al., Sci Rep, 2019
Exposure of F0 Pregnant Females

Tributyltin

GO enrichment [log(observed/5th-95th expected)]

Mouse GO distribution

Metabolic processes

%GC

Chromatin organization

GO enrichment [log(observed/5th-95th expected)]

Chamorro-Garcia et al., Nat Comms, 2017
Diaz-Castillo et al., Sci Rep, 2019

F0

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Self reconstructive propagation

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Tributyltin

Initial alteration

Self reconstructive propagation

Modes of action

Target tissues

- Tributyltin
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- Methoxychlor
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Chamorro-Garcia et al., Nat Comms, 2017
Diaz-Castillo et al., Sci Rep, 2019
Acknowledgments

Chamorro-Garcia Lab (UCSC)

Carlos Diaz-Castillo
Stephanie Aguiar
Tiffany Kluber
Prithvi Singh

Collaborators
Camilla Forsberg, UCSC
Diana Laird, UCSF

Blumberg Lab (UCI)

Bruce Blumberg
Riann Egusquiza
Bassem Shoucri
Heidi Kaech
Ron Leavitt

MGH-Harvard Med School
Toshi Shioda

INRA-Toxalim, Toulouse, France
Daniel Zalko