Defining DNA Methylation Signatures Associated with Metabolic Dysfunction in a Mexican American Cohort

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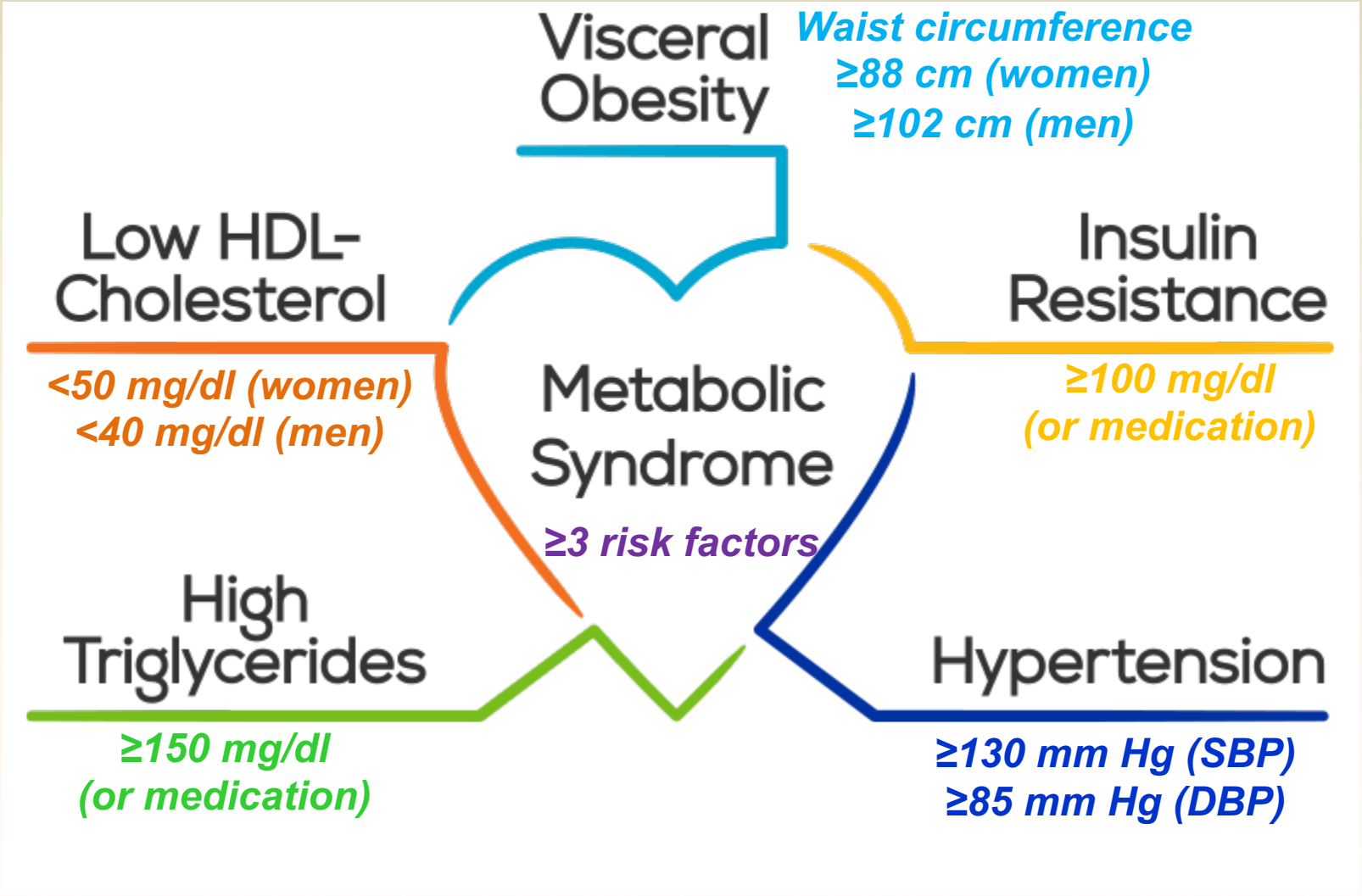
TEXAS BIOMEDICAL
RESEARCH INSTITUTE

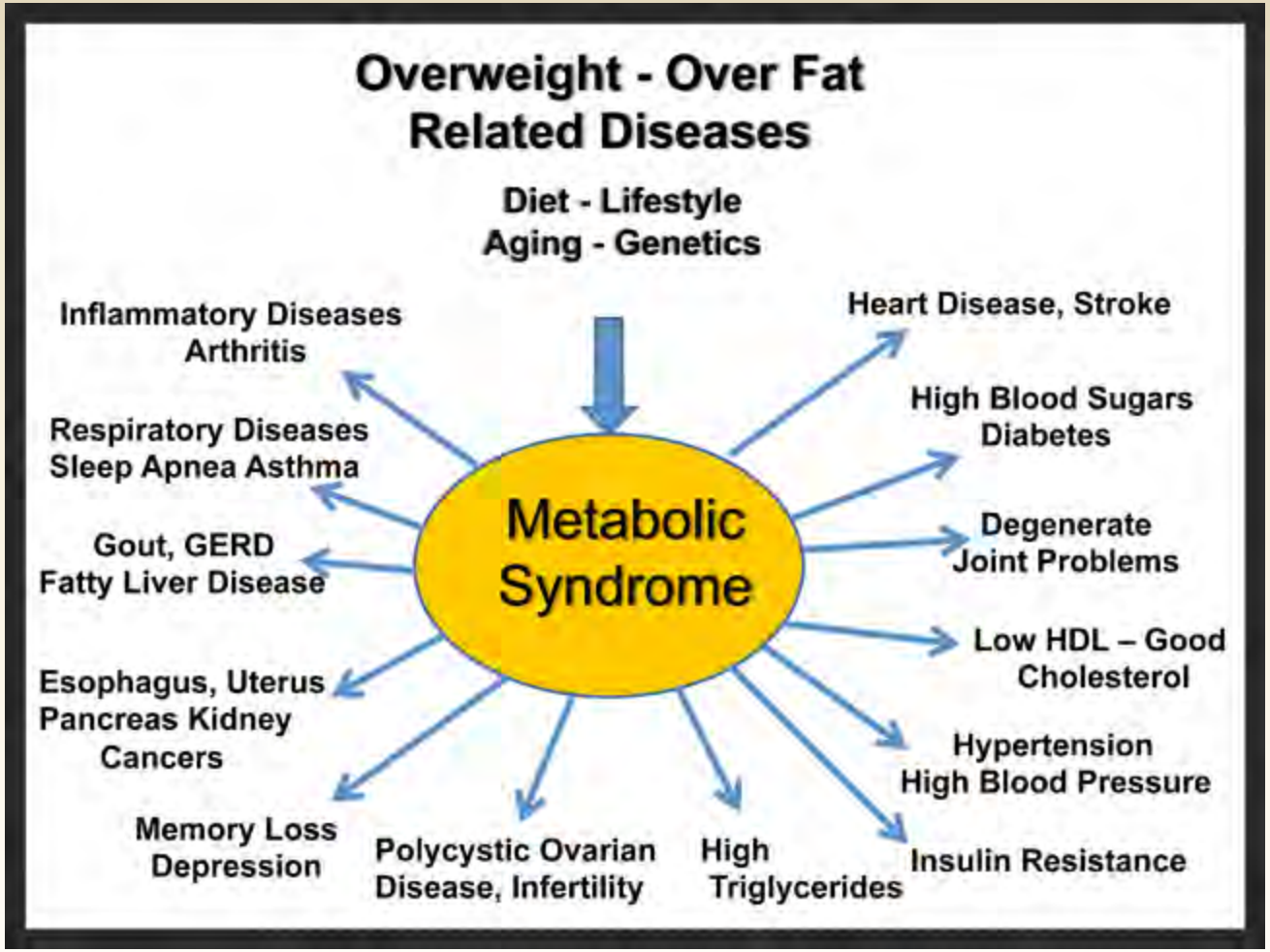
Metabolic Syndrome (MetS)

Risk Factors



https://www.metabolicsyndromecanada.ca/about-metabolic-syndrome (modified)

Metabolic Syndrome
Consequences



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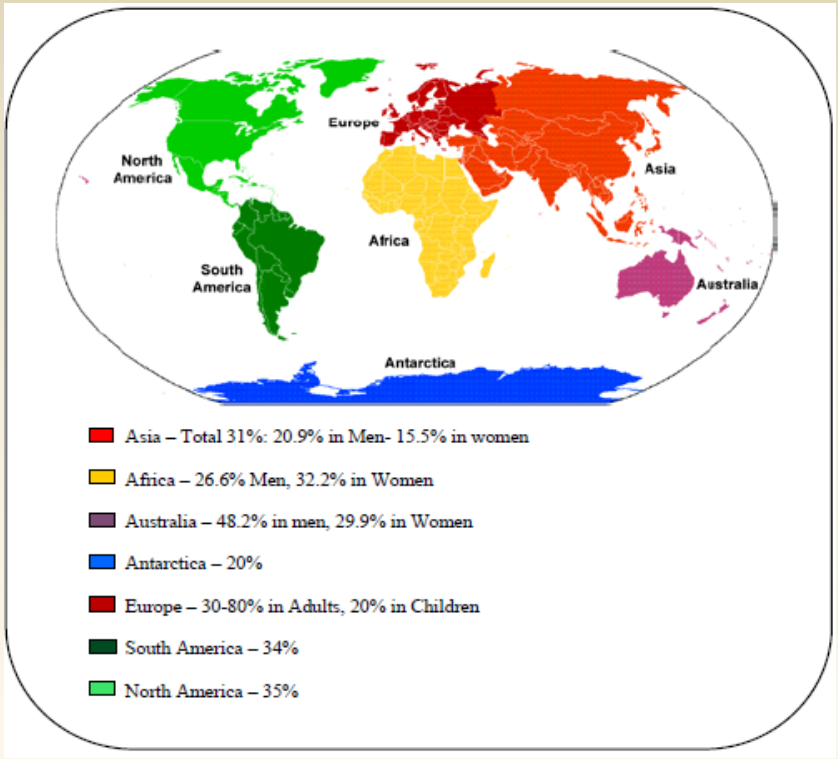
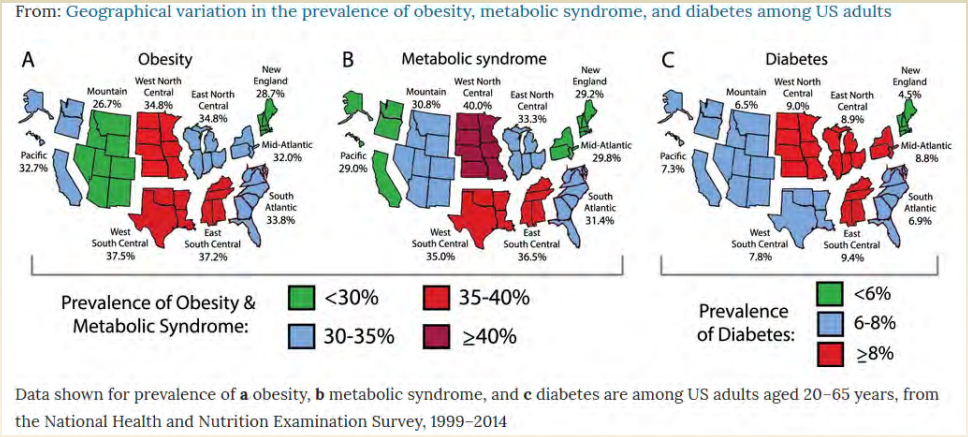
Metabolic Syndrome

Prevalence



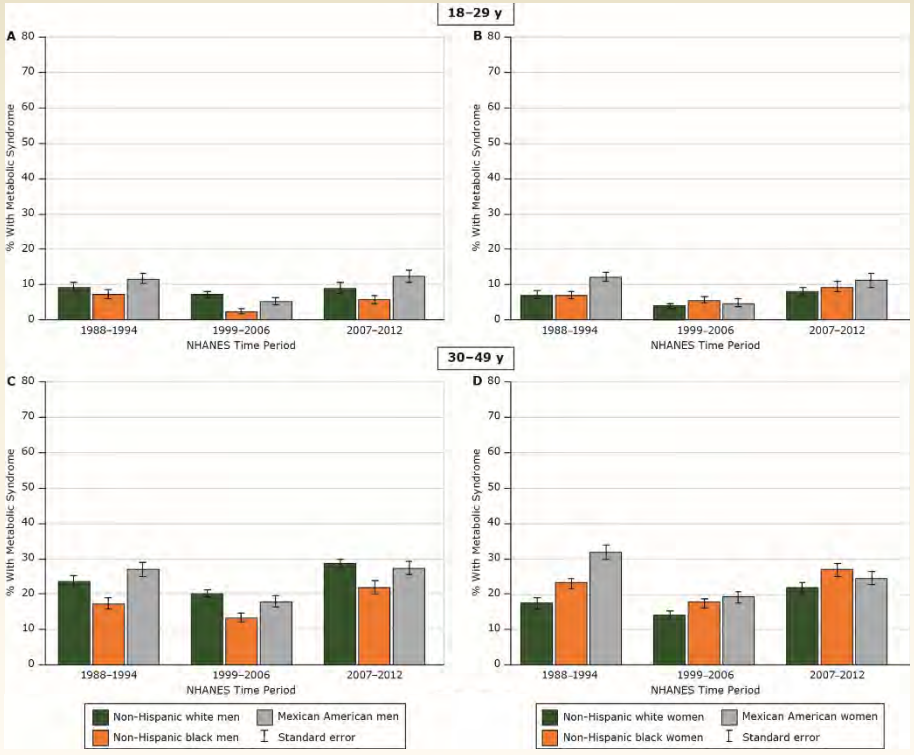
Pudata and Konduru (2011) *Journal of Diabetes and Metabolism*

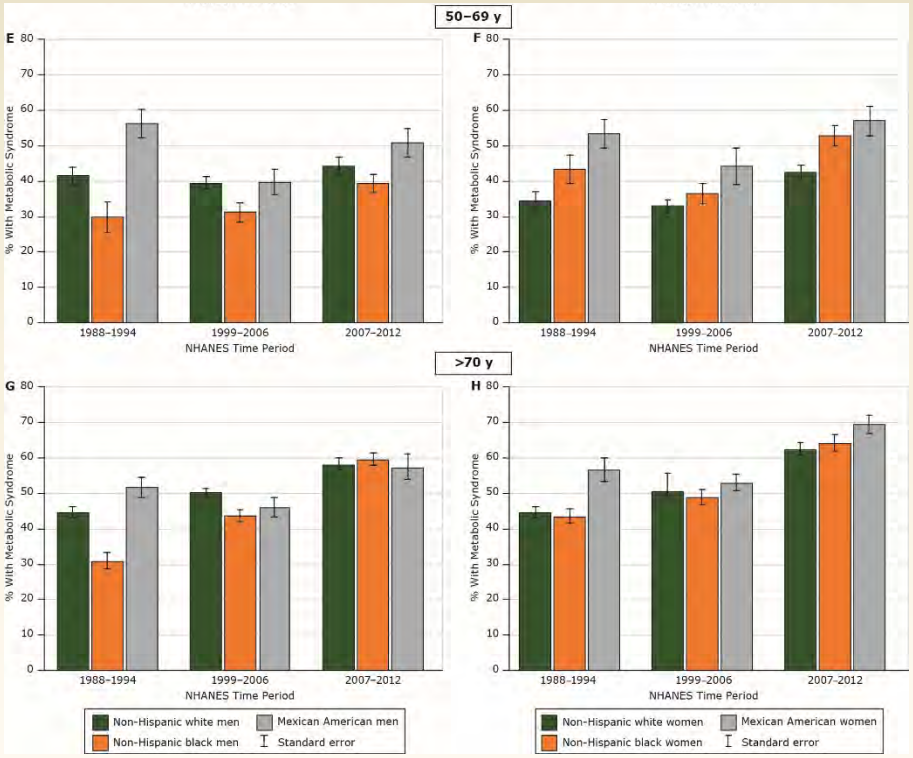


Gurka *et al* (2018) *Nutrition and Diabetes* **8**:14.

Metabolic Syndrome

At risk populations





Moore *et al* (2017) *Prev Chronic Dis* **14**:160287.

How did we get here? Where are we going?

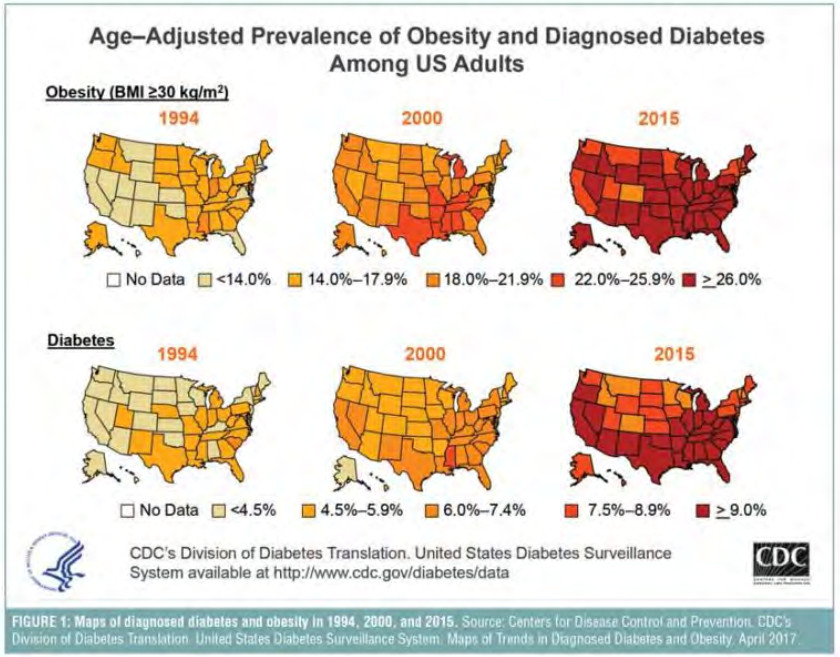


FIGURE 1: Maps of diagnosed diabetes and obesity in 1994, 2000, and 2015. Source: Centers for Disease Control and Prevention. CDC's Division of Diabetes Translation. United States Diabetes Surveillance System. Maps of Trends in Diagnosed Diabetes and Obesity. April 2017.

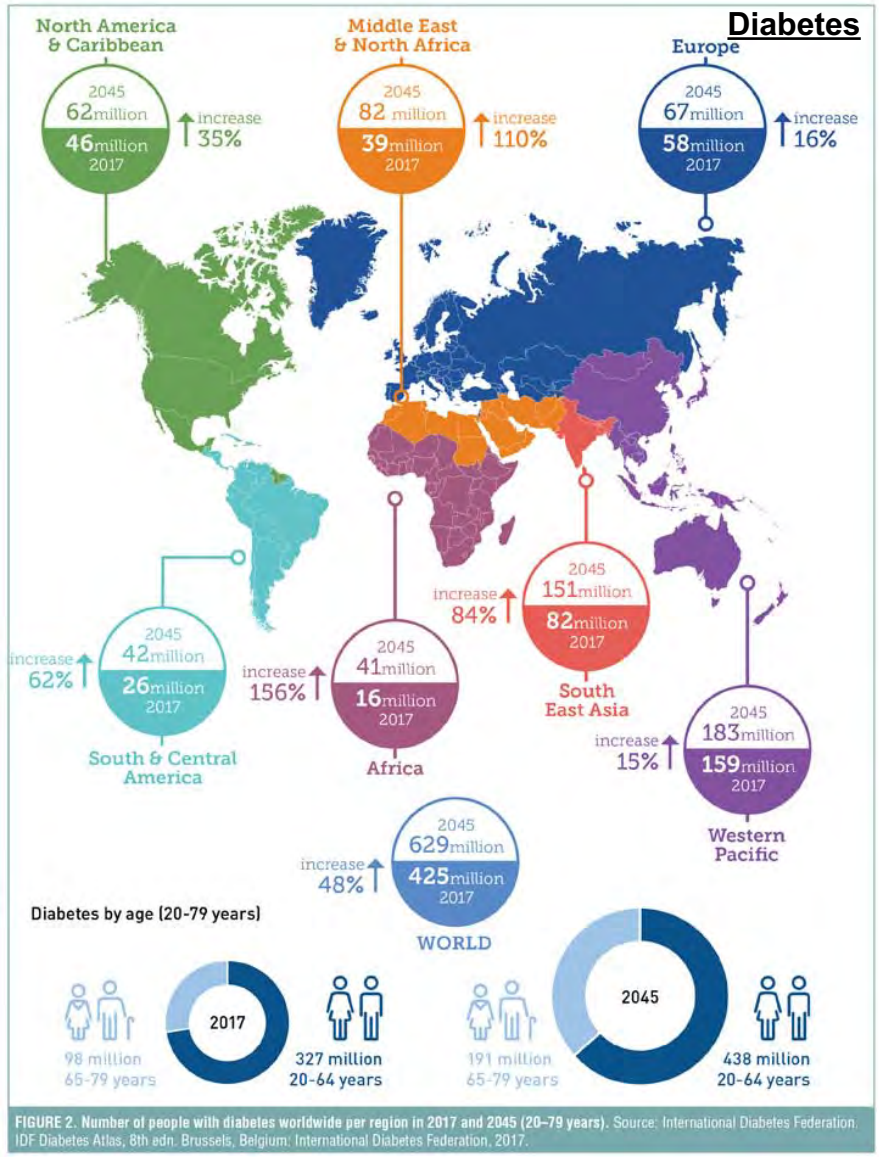


FIGURE 2. Number of people with diabetes worldwide per region in 2017 and 2045 (20–79 years). Source: International Diabetes Federation. IDF Diabetes Atlas, 8th edn. Brussels, Belgium: International Diabetes Federation, 2017.

http://bariatrictimes.com/diabesity-the-worldwide-twin-epidemics-of-obesity-and-diabetes/

San Antonio Family Heart Study

- Ongoing study of Mexican Americans to understand the genetics of complex diseases
  - Initiated in 1991 (to present); 5 collections
  - Large pedigrees (family-based study)
  - Extensive phenotypic data related to MetS

Trait	Visit 1	Visit 2	Visit 3	Visit 4
# Examined	1,431	859	950	1,378
Mean age at exam	39.3	42.9	47.5	44.8
Hypertension (%)	17.8	28.8	34.8	32.3
Obesity (%)	38.8	50.4	54.7	55.5
Type 2 diabetes (%)	15.1	19.7	21.3	20.1
Lipid lowering medication (%)	1.8	5.2	14.2	16.6
Metabolic Syndrome (%)		42.9	42.2	

PI: John Blangero

Why Epigenetics?

… genetic variants explain little of phenotypic variance

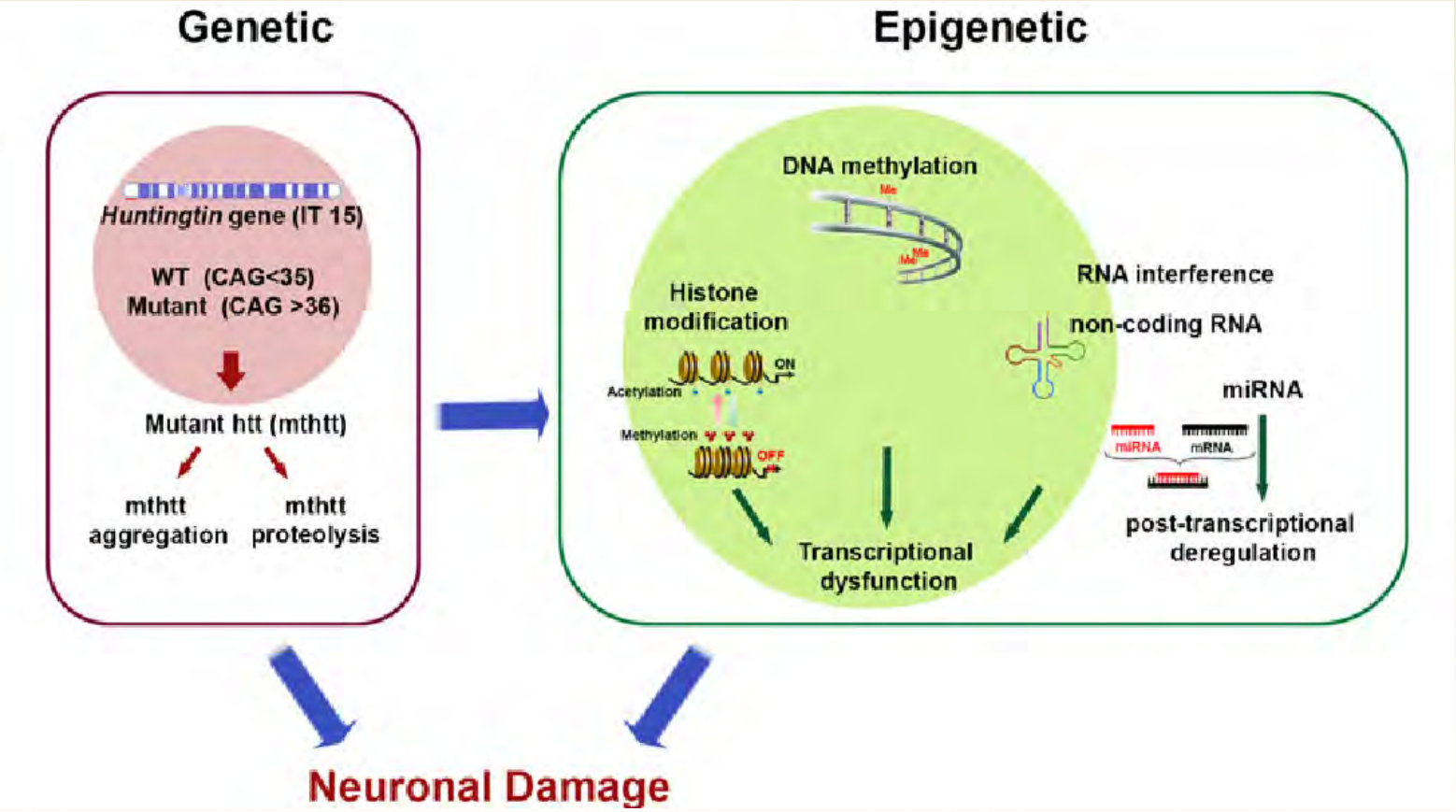
Trait	Heritability* (p-value)	GWAS-Explained Phenotypic Variance	Reference
Type 2 diabetes	0.61 (2.0×10^{-7})	17.5% (AA)	Ng *et al*, 2014. PLoS Genet, 10:e1004517.
Fasting glucose	0.42 (4.5×10^{-23})	4.8% (C)	Scott *et al*, 2012. Nat Genet, 44:991-1005.
Fasting insulin	0.42 (4.5×10^{-21})	1.2% (C)	
2hr glucose	0.37 (2.1×10^{-17})	1.7% (C)	
Total cholesterol	0.45 (4.6×10^{-28})	12.4% (C)	Teslovich *et al*, 2010. Nature, 466:707-13.
Triglycerides	0.49 (7.2×10^{-31})	9.6% (C)	
HDLC	0.54 (1.4×10^{-38})	12.1% (C)	
LDLC	0.36 (3.0×10^{-17})	12.2% (C)	
BMI	0.54 (5.9×10^{-37})	1.4% (C) (adj.) 16.5% (M) (all autosomal SNPs)	Shungin *et al*, 2015. Nature, 518:187-96. Yang *et al*, 2011. Nat Genet, 43:519-25.

* Heritability determined for the San Antonio Family Study cohort (Mexican Americans)

A: African American cohort
C: Caucasian cohort
M: Mixed race
adj.: Adjusted for waist hip ratio

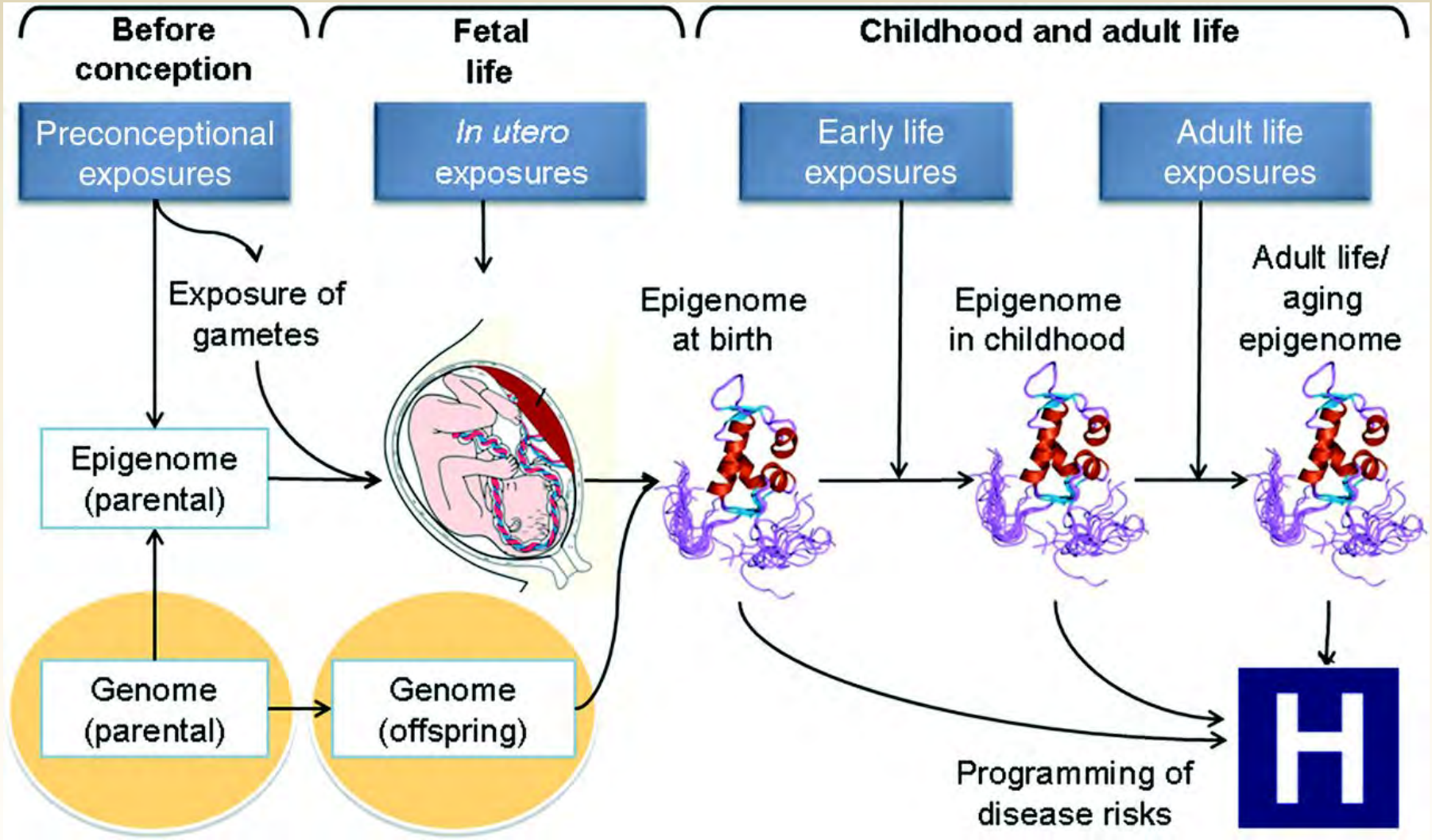
Epigenetics

Heritable phenotype changes that do not involve alterations in the DNA sequence
~ typically affects gene activity and expression



Lee *et al* 2013, *Neurotherapeutics* 10:664-76.

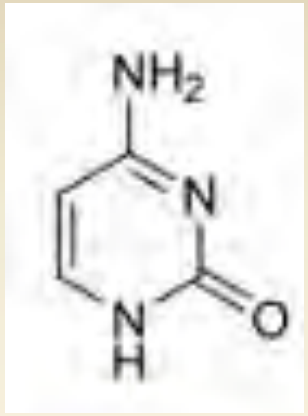
Why/When Does Epigenetics Matter?

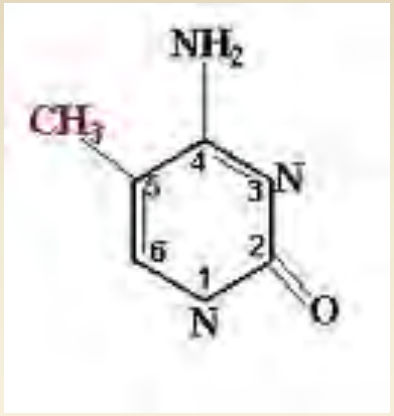


Fleisch *et al* 2012, *J Mol Endocrinol* 49:R61-7.

DNA Methylation

- Covalent addition of methyl group at C5 position of cytosines in cytosine-guanine (CpG) dinucleotides
  - Typically silences gene expression, but may increase expression, terminate transcription, or alter splicing
  - May predispose to additional mutational events





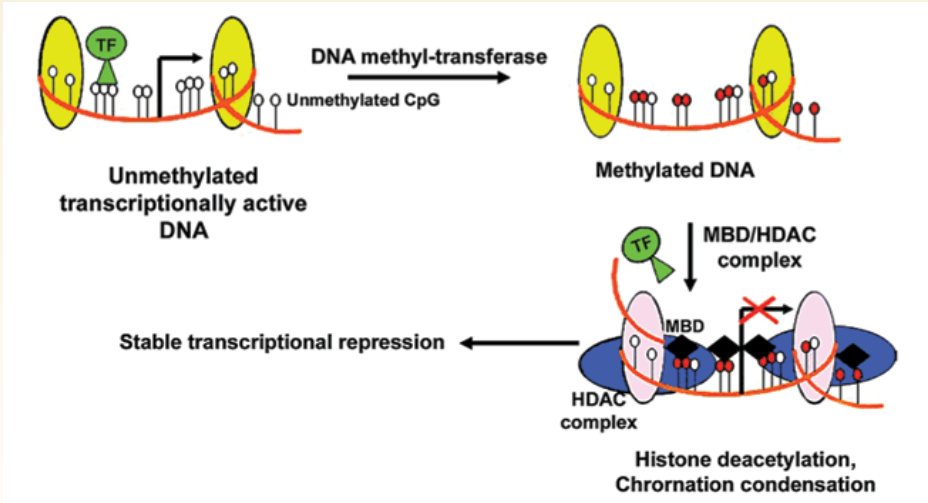


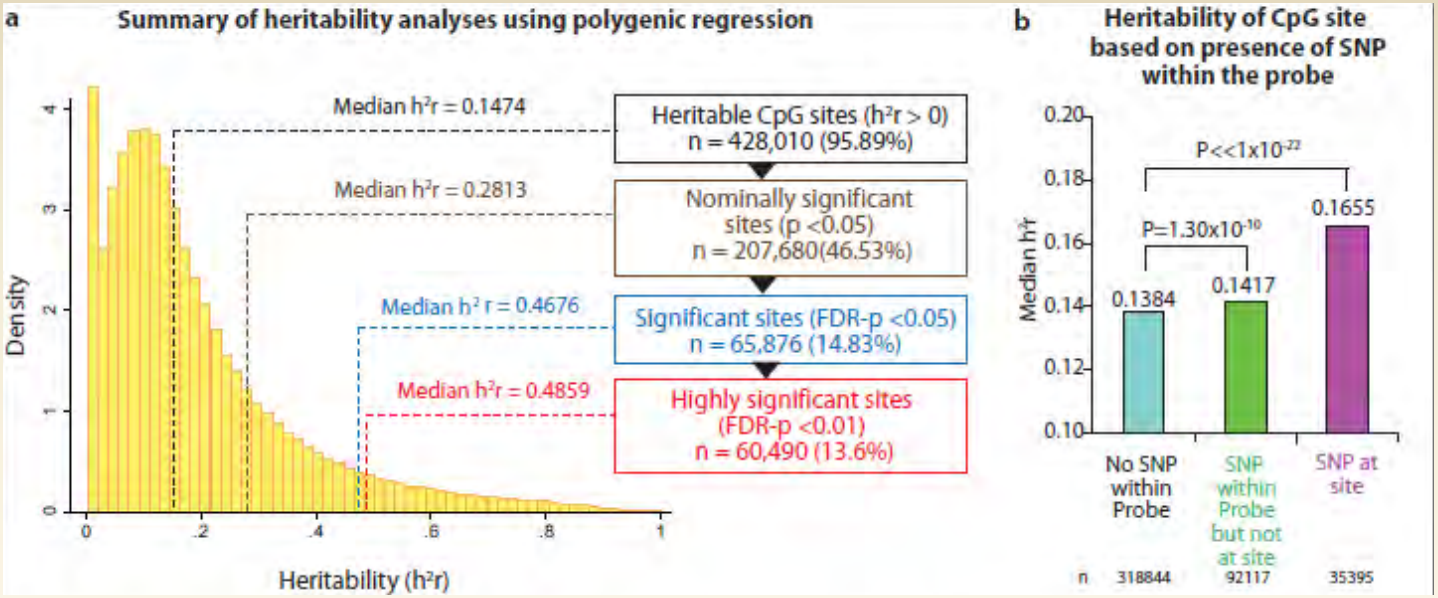
Figure 1 – Epigenetic silencing of gene expression. DNA methyl-transferases carry out the methylation of CpG dinucleotides, which triggers the process of gene silencing by recruitment of methyl binding domain (MBD) and Histone deacetylases (HDAC) to bind to the methylated DNA. This results in histone deacetylation and chromatin condensation leading to loss of transcription factor binding and subsequent repression of transcription.

Manoharan *et al*, 2007. Int Braz J Urol, 33:11-8.

Study Design

- Whole-genome methylation profiling (Illumina 450k BeadChips)
  - 859 individuals from visit 3
  - 341 individuals from visit 2
  - 241 of these overlap
- Normalization and quality control:
  - Probes normalized to control probes on array
  - Beta-mixture quantile normalization to correct probe bias
  - Inverse normalization to ensure normal distribution
  - Correction for cell counts, age, sex, medication, etc
- Assessed…..
  - Heritability of DNA methylation and meQTLs (Visit 3)
  - Age- and sex-associated CpG sites (Visit 3)
  - CpG sites associated with MetS risk (mostly Visit 3; Visit 2)

Heritability and Genetic Regulation of DNA Methylation



- meQTLs defined as ±50kb from CpG site
  - 14.3% of CpG sites are significantly associated with at least 1 SNP (average 10.3 significant SNPs per site)
  - Heritability was improved by 0.64% for each additional significant SNP
  - Number of significant methylation-SNP associations explained 12.47% of the variability in heritability

Kulkarni *et al* 2015, *Hum Mol Genet* 24:5330-44.

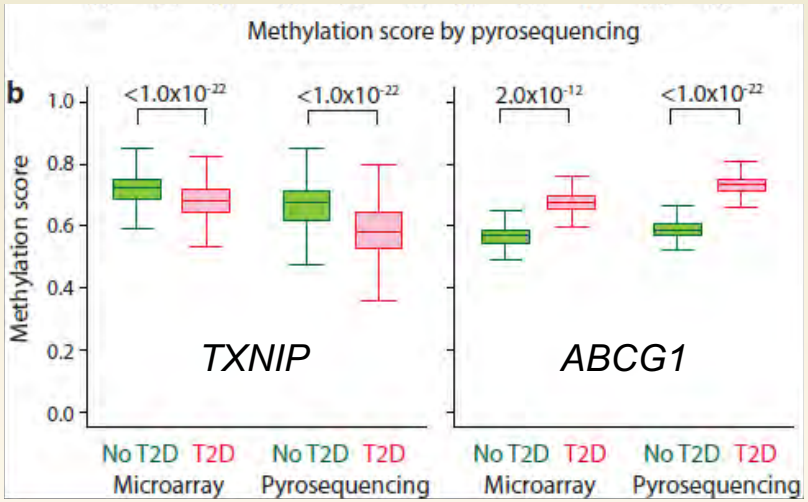
DNA Methylation is Associated with Age and Sex

- 22.3% of sites associated with age
  - 38.4% of these show increased methylation with age
  - Pathways associated: neurological disorders/neuronal system/neuronal transmission/long term potentiation, inhibition/regulation of insulin secretion, energy metabolism, cell communication and cell signaling pathways
- 2.8% of sites associated with sex
  - 85.6% of these hypermethylated in females
  - Pathways associated: nuclear receptor transcription, NOTCH and IGF1 pathways

Kulkarni *et al* 2015, *Hum Mol Genet* 24:5330-44.

DNA Methylation Influences Diabetes

- T2D/FG/HOMA-IR: 51/19/24 significantly associated sites
- 53 CpG sites had a composite significance score p<0.017
  - Top 5 associations accounted for 7.8% of the heritability of T2D (*TXNIP*, *ABCG1*, *SAMD12* and 2 intergenic sites)
  - CpG sites in *TXNIP* and *ABCG1* were validated by pyrosequencing

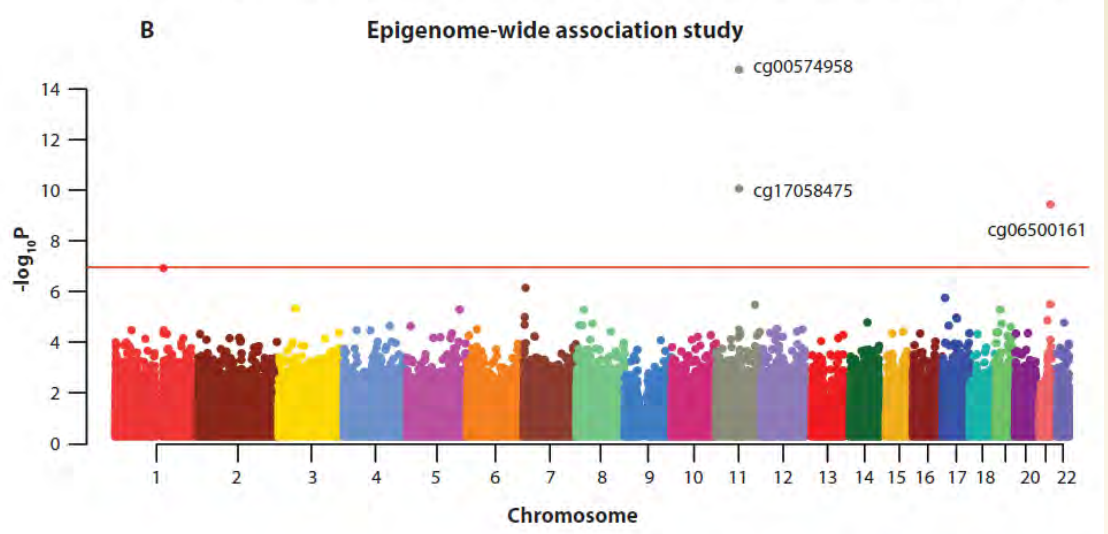


  - Biological candidates: *TXNIP*, *ABCG1*, *SREBF1*, *LOXL2*, *CPT1A*, *SOCS3*, *CALHM1*, *ICA1*, *ZBTB7A*, *CUX1*, *NFE2L3*, *LDLRAP1*
  - Gene enrichment analysis implicated pathways in insulin signaling, and those related to lipid signaling and transport

Kulkarni *et al* 2015, *Hum Mol Genet* 24:5330-44.

DNA Methylation Influences Obesity

- Hypertriglyceridemic waist (HTGW)
  - 3 significant associations (2 sites in *CPT1A*, 1 in *ABCG1*)
    - Accounted for 9.52% of the variability in HTGW
    - Top *CPT1A* site validated by pyrosequencing and also associated with Visit 2 data



Mamtani *et al* 2016, *Clin Epigenetics* 8:6.

- Obesity measures
  - BMI: 8 associations
  - WC: 3 associations
  - Obesity: 12 associations
  - Biological candidates: *APOL1*, *CPT1A*, *NOD2*, *PHGDH*, *SOCS3*, *TNFSF10*

DNA Methylation Influences Lipids and Blood Pressure

- Lipid Phenotypes
  - Total cholesterol: 2 significant associations
  - HDLC: 32 significant associations
  - LDLC: 11 significant associations
  - Triglycerides: 10 significant associations
  - Biological candidates: *CPT1A*, *TXNIP*, *CALHM1*, *ABCG1*, *TNIP2*, *KLF13*, *KCNQ1*

- Blood pressure
  - Only suggestive significance: *CAMTA1*, *GALNT2*, *NOTCH4*

MetS-Associated DNA Methylation Changes

- Metabolic syndrome
  - 3 significant associations: *CPT1A*, *TXNIP*, *ABCG1*

- *TXNIP*: Glucose/insulin tolerance; triglyceride concentration in diabetics; mediates diet-induced obesity[1-3]
- *CPT1A*: Involved in fatty acid oxidation, implicated in regulation of feeding[4], obesity and lipid levels (methylation)[5-7]
- *ABCG1*: Involved in glucose and lipid homeostasis[8], failure to identify genetic variation associated with T2D[9], methylation within this gene implicated in obesity[6], plasma lipid levels[10] and insulin resistance and diabetes[11-12]

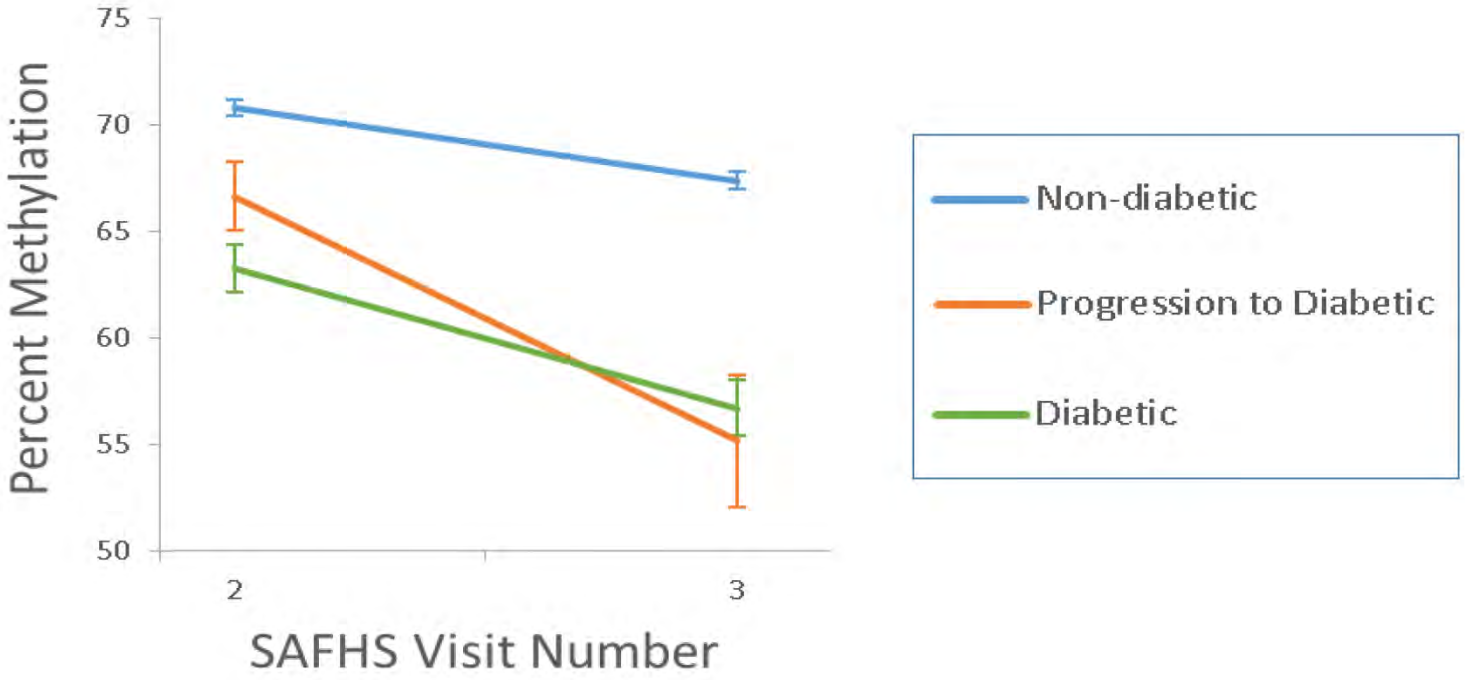
1. Jo *et al* 2013, *Diabetolgia* 56:2723-32.
2. van Greevenbroeck *et al* 2007, *Diabetes Med* 24:498-504.
3. Blouet *et al* 2012, *J Neurosci* 32:9870-7.
4. Mera *et al* 2014, *PLoS One* 9:e97195.
5. Aslibekyan *et al* 2015, *Obesity* 23:1493-501.
6. Demerath *etal* 2015, *Hum Mol Genet* 24: 4464-79.
7. Gagnon *et al* 2014, *J Lipid Res* 55:1189-91.
8. Mauldin *et al* 2008, *Circulation* 117:2785-92.
9. Schou *et al* 2012, *Diabetes Care* 35:2600-6.
10. Pfeiffer *et al* 2015, *Circ Cardiovasc Genet* 8:334-42.
11. Hidalgo *et al* 2014, *Diabetes* 63:8101-7.
12. Chambers *et al* 2015 *Lancet Diabetes Endocrinol* 3:526-34.

DNA Methylation Changes Associated with Development of Diabetes and Obesity

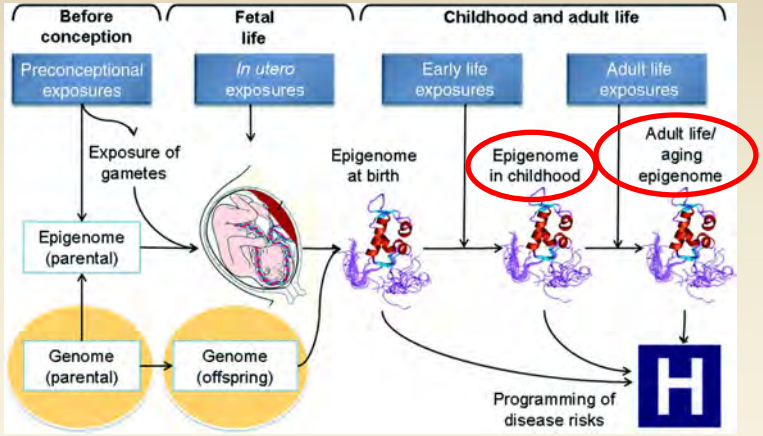
- Assessed candidate regions for differences in DNA methylation over time
  - Visit 2 vs visit 3; n=241 individuals
  - *TXNIP*, *ABCG1*, *CPT1A*, *SREBF1*, *LOXL2*, *SOCS3*, *CALHM1*

Phenotype	Gene	P-value
$Fasting\ Glucose_{Diff}$	TXNIP CPT1A LOXL2	$1.07x10^{-4}$ $1.28x10^{-3}$ $2.86x10^{-3}$
$Obesity_{Diff}$	ABCG1	$4.47x10^{-3}$
$Waist\ Circumference_{Diff}$	CPT1A	$5.33x10^{-3}$
$\%\ Body\ Fat_{Diff}$	CPT1A	$6.78x10^{-3}$

DNA Methylation changes in *TXNIP* may Precede Diabetes Onset



What Next?
Early Intervention???

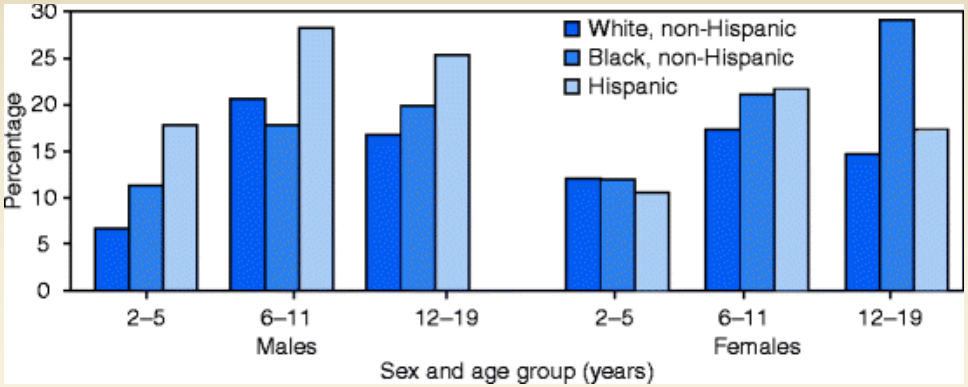


Prevalence of metabolic syndrome varies greatly in children, based on diagnostic criteria

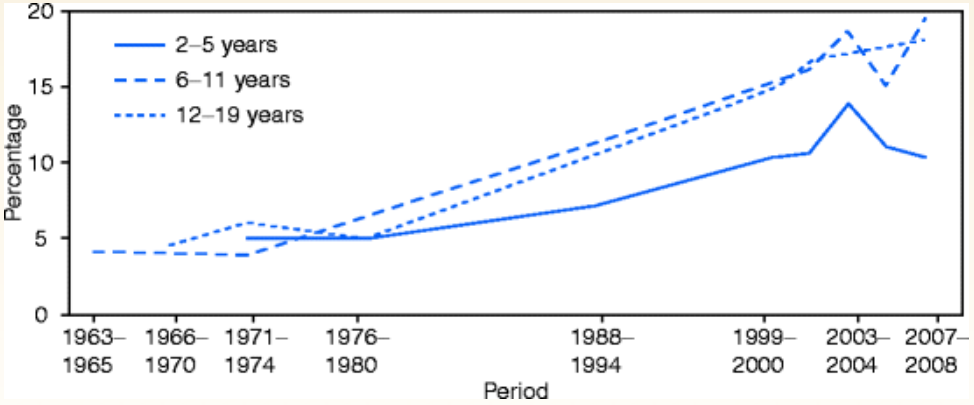
- Median prevalence in non-obese/non-overweight children 0-1%
- Median prevalence in overweight children 2.8-29.3%
- Median prevalence in obese children 10-66%

Friend *et al* (2013) *Metab Syndr Relat Disord* **11**:71-80.

Prevalence of obesity in children

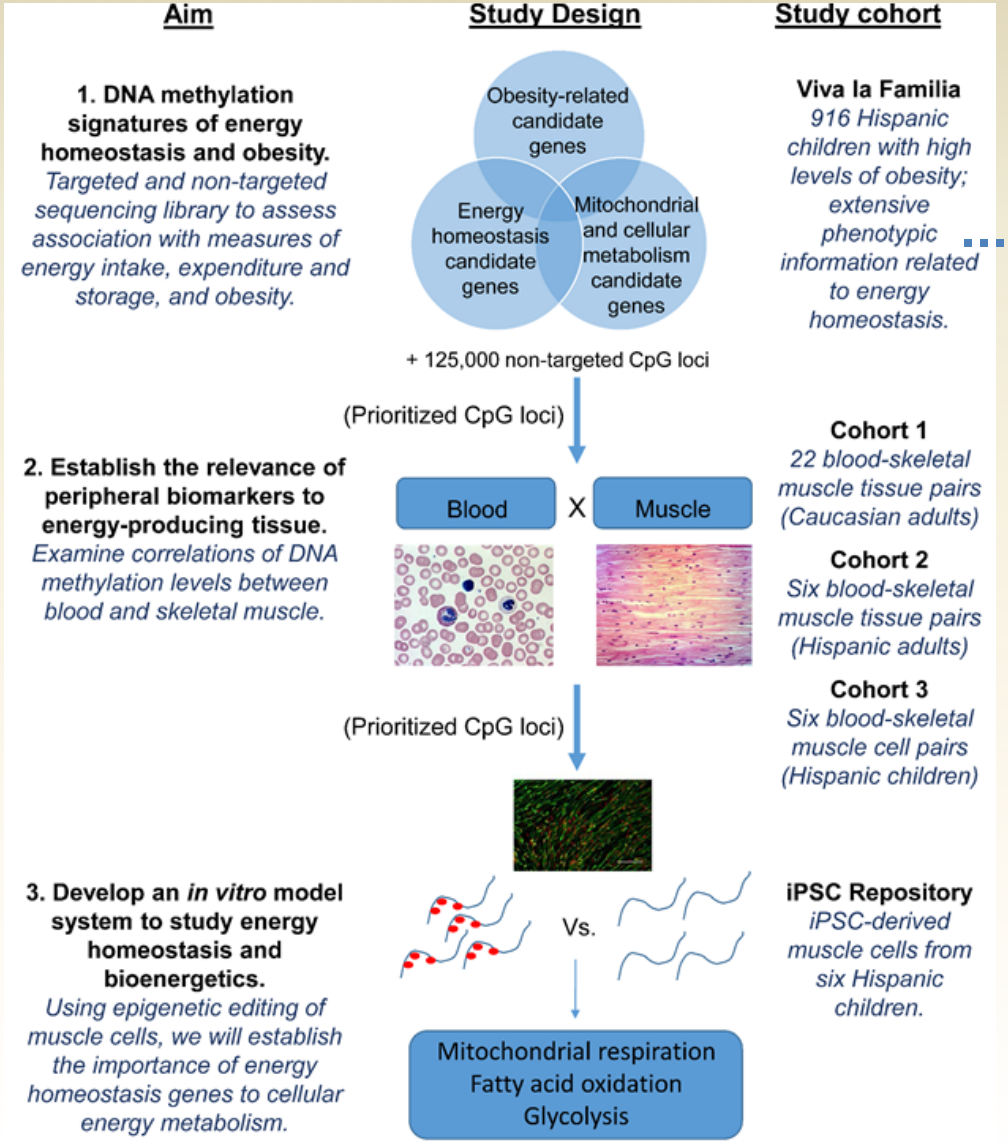


Prevalence of obesity among children and adolescents by sex, age group, and race/ethnicity, United States, 2007–2008 (Reprinted from Centers for Disease Control and Prevention)



Messiah *et al* (2012) in *Pediatric Metabolic Syndrome* pp37-55.

Focus on obesity/energy homeostasis nexus



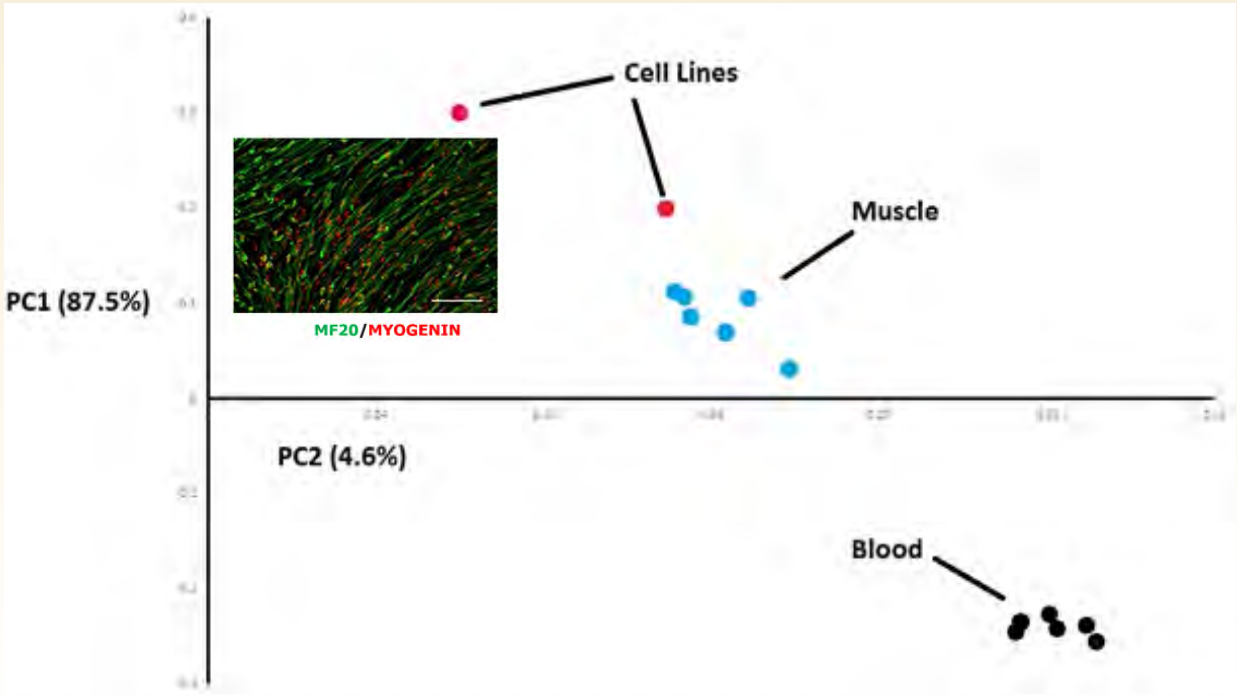
Viva la Familia (VIVA) study

PI: Shelley Cole/Nancy Butte

Anthropometry
Body composition
Energy intake
Total energy expenditure
Basal metabolic rate
24 hour respiratory quotient
Protein/fat oxidation
Physical activity (accelerometers)

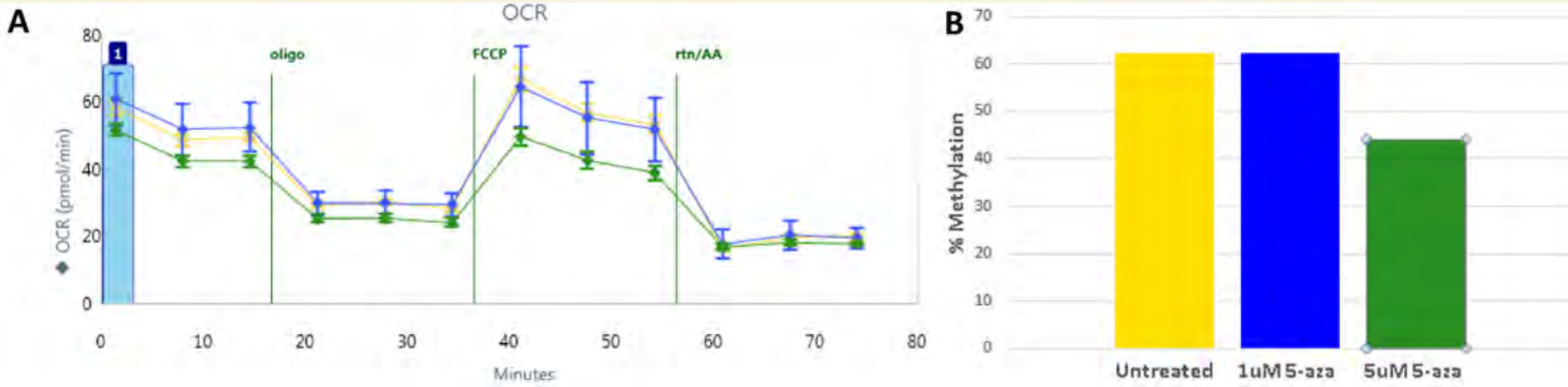
DNA Methylation in Muscle

- Whole-genome methylation sequencing
  - Overall correlation between blood and muscle 0.84-0.89 ($p=2.2x10^{-16}$)
  - PCA analysis indicates similarity between ES/iPSC-derived muscle cells and muscle tissue

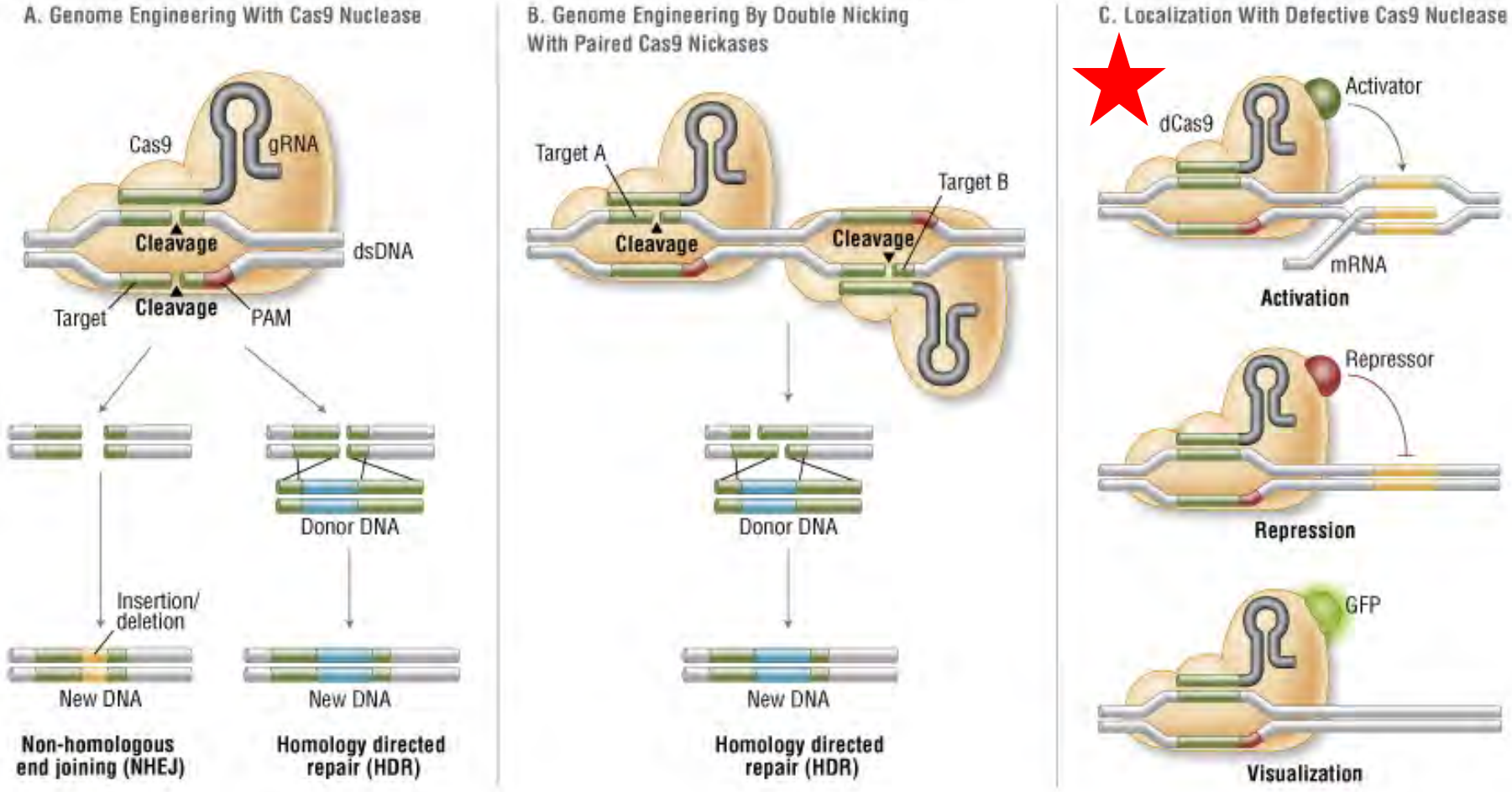


DNA Methylation and Mitochondrial Function

- Seahorse mitochondrial stress test assay
  - Human skeletal muscle cells treated with 5-azacytidine
  - Global demethylation decreases basal (86%) and maximal (66%) respiration, ATP production (91%), and proton leak (70%) compared to control cells



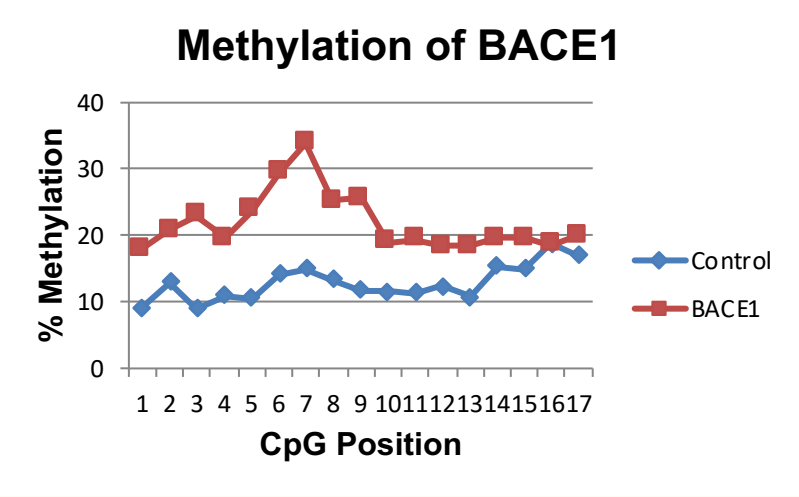
CRISPR: Cas9 Variants

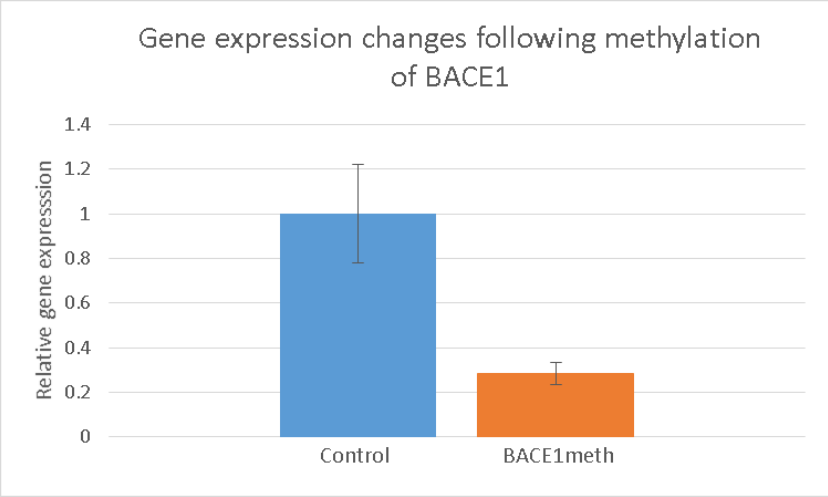


https://www.neb.com/tools-and-resources/feature-articles/crispr-cas9-and-targeted-genome-editing-a-new-era-in-molecular-biology

Stable Methylation of iPSCs

- To bypass editing of terminally differentiated muscle cells, we developed a platform to alter DNA methylation in iPSCs at specific sites using CRISPR-dCas9 system
  - Generated stable iPSC lines for methylated *BACE1* promoter using 3rd generation lentiviral system (methylation retained > 40 days)
  - Associated with a significant decrease in gene expression





- Will use this system to understand function of altered methylation related to obesity and energy homeostasis

Collaborators

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University of Alabama
Bertha Hidalgo
Howard Wiener

Baylor College of Medicine
Nancy Butte

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Dawn Coletta

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Looking for postdocs
Expertise in stem cells, genetic/epigenetic editing, and/or data analysis/bioinformatics!!!