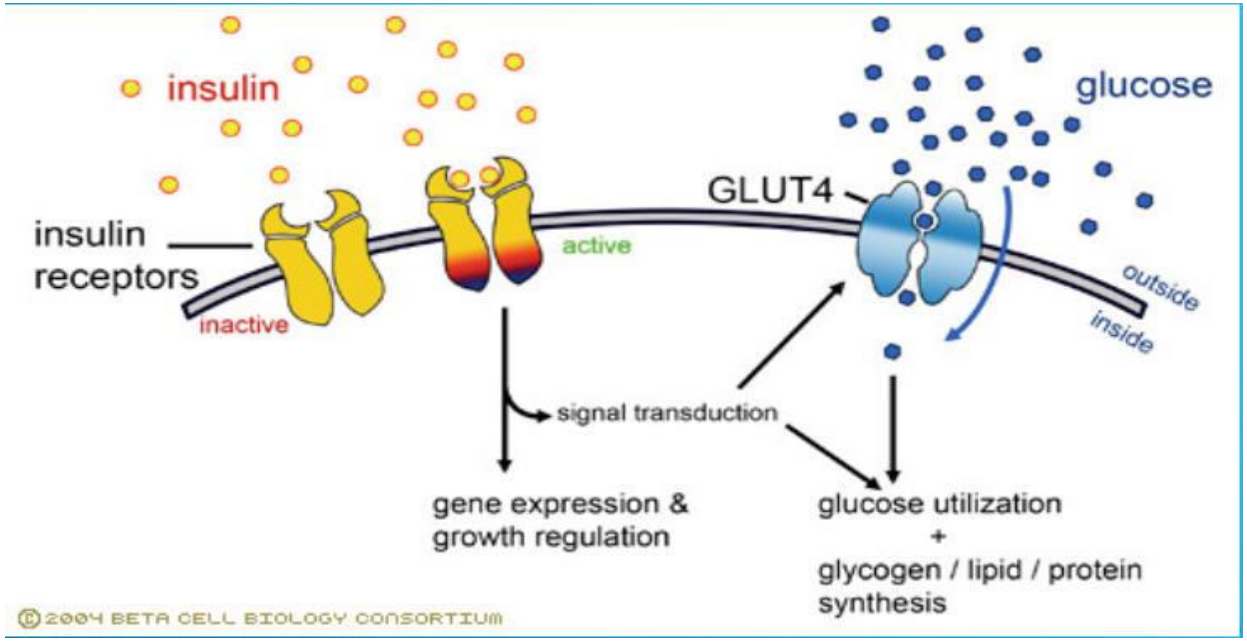
Genome-wide gene expression analysis in black South African women who develop gestational diabetes mellitus

Angela Steyn (MSc MED)
Supervisors: Prof Michele Ramsay and Prof Shane Norris
University of the Witwatersrand, Johannesburg, South Africa

Introduction - Diabetes

- Metabolic disorder with a myriad of abnormalities affecting hormones and nutrient substrates

- Body fails to either produce insulin (Type 1 diabetes) or utilize insulin correctly (Type 2 diabetes)



Gestational diabetes

- Pregnant women exhibit high blood glucose levels in their blood -first recognition or onset in pregnancy
- No prior diagnosis of diabetes
- Industrialized countries (every 10th pregnancy affected); diagnosed by OGTT at 24 – 28 weeks gestation

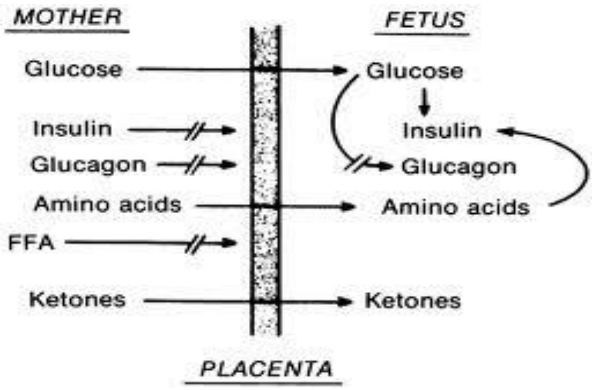
Gestational diabetes mellitus	
	Venous plasma glucose treshold (mmol/L)
Fasting	≥ 5.1
75 g oGTT: 60 min	≥ 10.0
75 g oGTT: 120 min	≥ 8.5
One or more values equal or exceeding diagnostic threshold.	
Overt diabetes in pregnancy	
Measure of glycaemia	Diagnostic threshold
Fasting plasma glucose (FPG)*	≥ 7.0 mmol/L
HbA_{1c}	≥ 6.5% (48 mmol/mol)
Random plasma glucose*	≥ 11.1 mmol/L
Any of measures of glycaemia equal or exceeding diagnostic threshold.	
* venous plasma	

Gestational diabetes

- Transient form of disease
- Predisposes mother to high risk of developing T2D
- Predispose fetus to adult onset diseases, increased incidence of macrosomia; fetal death, childhood obesity
- Routine screening
- Early intervention and possible treatment – insulin; diet; exercise

The Physiology of pregnancy

- The **diabetogenic effect** observed during pregnancy is due to an increase in the demand for insulin in later stages of pregnancy
- The exact mechanism responsible for this state of insulin resistance is uncertain



- State of insulin resistance is required – ensure glucose reaches developing fetus (main energy substrate for growth)
- Insulin doesn't cross placenta

Glucose metabolism in healthy pregnancy

Placental hormones increase
Lactogen
Progesterone
Cortisol

Partially block action of insulin

Insulin resistance increases

Increasing food supply to fetus during late pregnancy creates diabetogenic effect

Pancreatic β-cell effect

To compensate for the insulin resistance; pancreatic β cells increase their insulin secretion

This flexibility of β cell function is the hallmark for normal glucose regulation during pregnancy

Normal glucose tolerance

Glucose metabolism in Gestational diabetes

Partially block action of insulin

Insulin resistance increases

Increasing food supply to fetus during late pregnancy creates diabetogenic effect

Pancreatic β-cell deficiet

maternal β-cell function can not adapt to increased insulin demand of late pregnancy

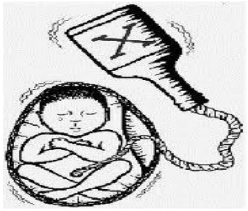
Some pregnant women are unable to up regulate insulin production relative to the degree of insulin resistance

Gestational diabetes

Increasing blood glucose levels

Epigenetics

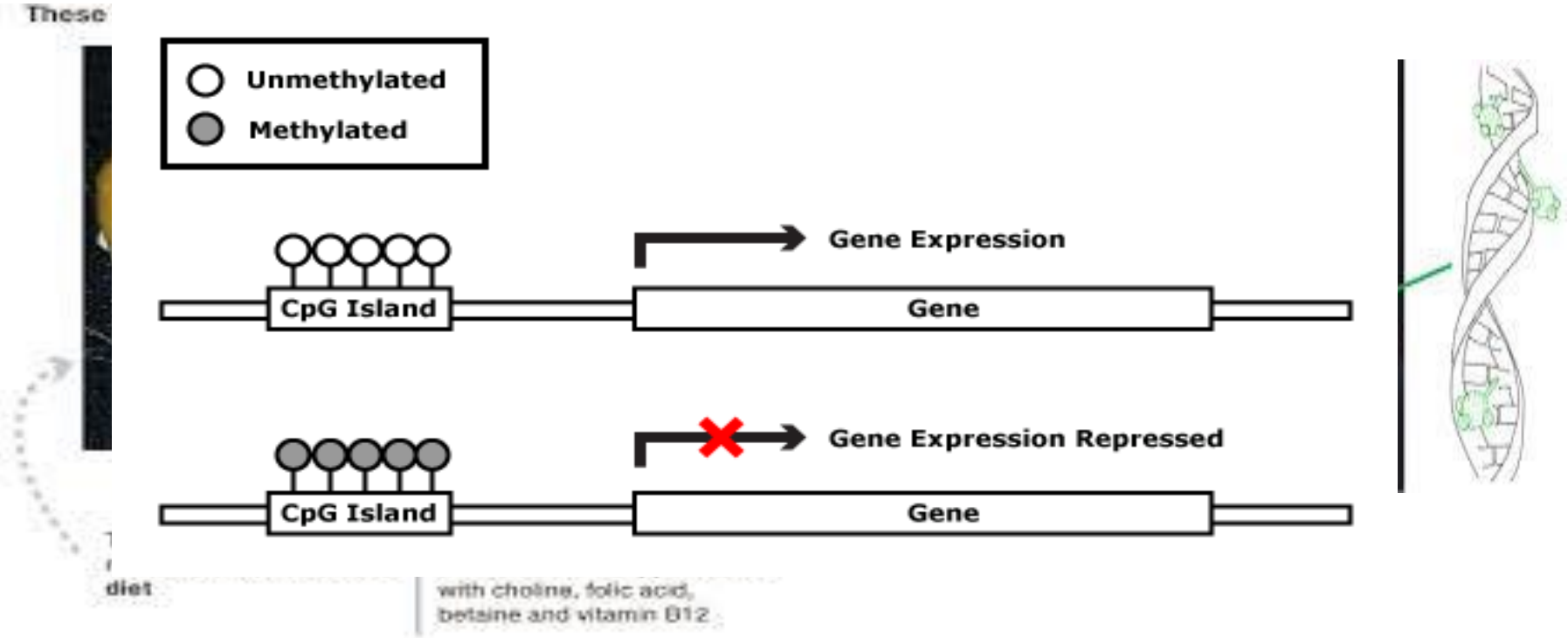
- is the study of heritable changes in gene activity that are *not* caused by changes in the DNA sequence (Histone modification, non coding RNAs and DNA methylation)
- Adverse intrauterine environment may play a critical role in determining fetuses susceptibility to developing chronic diseases



- Adapt to ensure survival under conditions exposed too (too little nutrients, too much nutrient etc)

- Adaptations thought to act through epigenetic changes – which are established early in life and control expression of certain genes during development

Agouti Mice


These
Unmethylated
Methylated
CpG Island
Gene Expression
Gene
CpG Island
Gene Expression Repressed
Gene
diet
with choline, folic acid,
betaine and vitamin B12

Dutch Hunger Winter

Dutch Famine Birth Cohort Study- prospectively studies offspring of mothers who were exposed to the famine

Offspring exposed were on average smaller – 60 years later they have a higher prevalence of adult onset disease

Epigenetics studies - HYPOMETHYLATION of IGF2 gene

The major role of IGF-2 is as a growth promoting hormone during gestation.

AIM and OBJECTIVES

To understand the impact of gestational diabetes on the fetus by investigating gene expression and DNA methylation to look for epigenetic perturbations that may lead to an unfavorable outcome in the offspring.

To assess differential genome-wide gene expression in:

1. Blood samples from women with GDM and controls.

2. Placental tissue biopsy samples from female/male neonates born to women with GDM (adverse in-utero environment) and those born to controls (healthy in-utero environment; absence of GDM).

To examine DNA methylation patterns in the subset of selected genes that show a significantly altered differential expression (≥2 fold).

Materials and Methods

Sample Collection

IDAPSG criteria
Anthropometric data

RNA and DNA extraction and QC

HumanHT12v4 bead chip

Extension bioinformatic analysis of data

Select genes which show significant differential expression

Confirmatory RT-PCR SYBR green experiment

Identify genes for methylation studies

- Design custom EpiTect Methyl II Custom PCR methylation Array for selected genes

- Analysis using Bioinformatic algorthims

Materials and Methods

GDM and controls for Angela PhD								
Lab code	Case/Co	Proposed Visit	Consent	Two blood tubes	Expected delivery date	Placenta biopsy		
1054	case	10-04-14	yes	yes	10-05-14	yes		CASE #1
1056	control	11-04-14	yes	yes	11-05-14	yes		CONTROL #1
1050	control	16-04-14	yes	yes	03-06-14	missed	did not	out of study
1045	control	23-04-14	yes	yes		missed	different	out of study
1048	case	25-04-14	yes	yes		yes		CASE #2
1060	case		yes	yes				
1061	control		yes	yes				
1063	control		yes	yes				
1066	control	01-07-14						
1068	control	01-07-14						

Anthropometric data (weight, length); Mother age; previous pregnancies; mothers age; mothers BMI etc

Possible findings

- **Comparing GDM woman to healthy controls (using venous blood)**
- Identify genes that display a significant change in expression between groups

- **Comparing fetal environments (using placenta)**
- Identify genes that display a significant change in expression between an adverse (GDM) intra-uterine environment and a healthy one.

Find common genes that

- Show significant differential expression in both case groups (GDM women and GDM placenta)
- Have CpG island in promoter region

After DNA methylation analysis of these selected genes

- The change in gene expression may be a result of altered DNA methylation in the genes promoter region
- This altered methylation pattern is observed in placenta as well