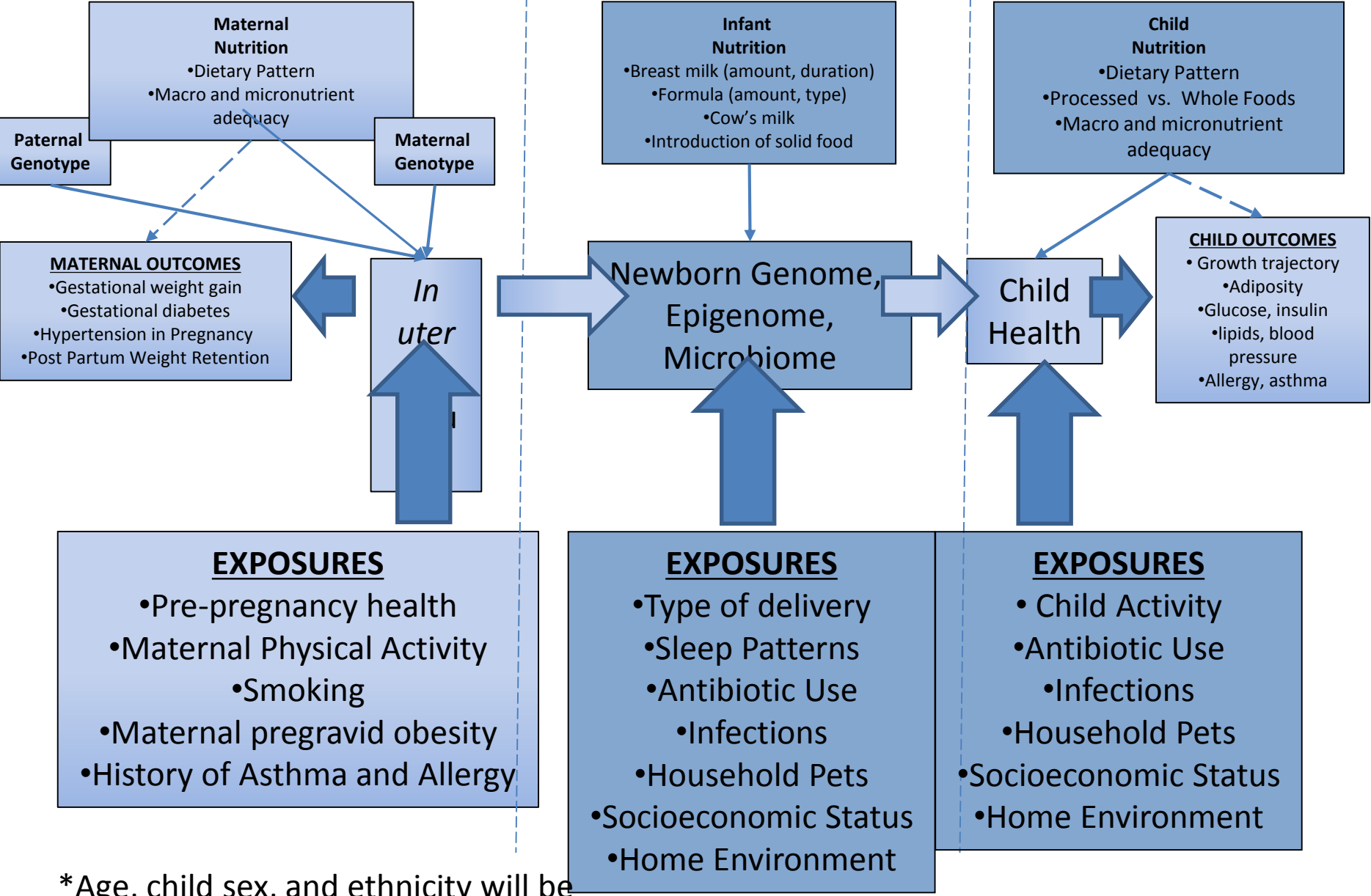
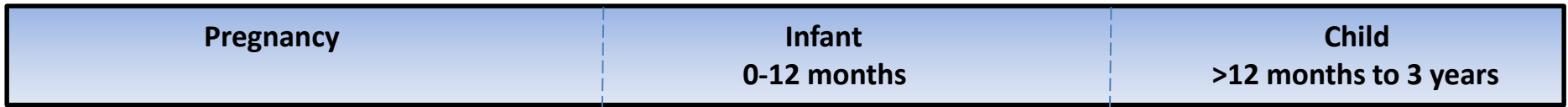


# Nutrition Grant Overview

Sonia Anand

# We proposed in Diverse Populations:

- 1) Systematic Reviews of Maternal dietary intake and infant outcomes
- 2) Maternal nutrition as related to newborn, infant and child health outcomes (Primary data analysis)
- 3) Gene-diet interactions, Epigenetic patterns in cord blood in maternal diet subgroups
- 4) Microbiota profile in Infants age 1 linked to infant diet + health outcomes



\*Age, child sex, and ethnicity will be

# Cohort Harmonization

CHILD	FAMILY	START	ABC	TOTAL
3,600	900	1,000*	300*	5800

\* Recruitment on-going but should be completed by 2015

# Dietary and Biological Measures

	Child	Family	START-Canada	ABC
Maternal FFQ	Fred Hutchison	SHARE	SHARE	SHARE
Infant feeding Assessment	Yes	Yes	Yes	Yes
Maternal DNA	Yes	Yes	Yes	Yes
Cord Blood or infant DNA	Yes	Yes	Yes	Yes
Socioeconomic Characteristics	Yes	Yes	Yes	Yes
Maternal physical Activity	Yes	Yes	Yes	Yes
Infant/Child activity	Yes	Yes	Yes	Yes
Prospective Follow-up	Yes	Yes	Yes	Yes
Record Linkage to Provincial Databases	Yes (Ontario)	No	Yes	Yes
Microbiome Sample	Yes	No	Yes	Yes
Breast Milk Sample	Yes	No	Yes	Yes
Infant Adiposity at birth		Yes	Yes	Yes
Infant Adiposity at 1 or 3 years	Yes	Yes	Yes	Yes
Infant wheeze	Yes	Yes	Yes	Yes
Infant spirometry	Yes	No	No	No
Infant Allergy Testing	Yes	Yes	TBD: 500	TBD

# Design: Measurement, Outcomes, and Power

Exposure	Maternal Outcomes	Birthoutcomes	Infant-1 yr	Child 3 vs 5 years
Maternal Diet	Weight Gain <sup>2,3,4</sup>	Birth weight <sup>1,2,3,4</sup>	Growth <sup>1,2,3,4</sup>	Growth <sup>1,2,3,4</sup>
*FFQ' s collected in pregnancy from all mothers	Gestational DM <sup>1,2,3,4</sup>	Adiposity <sup>2,3,4</sup>	Adiposity change since birth <sup>1,2,3,4</sup>	Adiposity change since birth <sup>1,2,3,4</sup>
*Nutritional Supplements	Hypertension in Pregnancy <sup>1,2,3,4</sup>	Premature Delivery <sup>1,2,3,4</sup>	Metabolic traits (Glucose <sup>3,4</sup> , Lipids <sup>3,4</sup> , Blood pressure <sup>2,3,4</sup> )	Metabolic traits (Glucose, Lipids, Blood pressure) <sup>2,3,4</sup>
*Confounder variables: - Smoking, Drug Use	Post partum weight retention <sup>1,2,3,4</sup>	? Others	Allergy –Skin Test <sup>1,2</sup>	Allergy –Skin Test <sup>1</sup>
	Asthma <sup>1,2,3,4</sup>		Asthma “self report” <sup>1,2,3,4</sup>	Asthma “Self Report” <sup>1,2,3,4</sup>
	Allergy <sup>1,2,3,4</sup>		Atopic Disorders “self report” <sup>1,2,3,4</sup>	Atopic Disorders “Self Report” <sup>1,2,3,4</sup>

Collected in CHILD<sup>1</sup>, FAMILY<sup>2</sup>, START<sup>3</sup>, ABC<sup>4</sup>

# Baseline Characteristics

	Child	Family	START-Canada
N		692	251
Maternal Age (SD)		32.11 (4.99)	29.90 (4.01)
White Caucasian (%)	71.4	87	0
Ontario (%)		100%	100%
Pre-pregnancy weight (SD)		72.90 (17.85)	62.23 (12.93)
Maternal Weight Gain		14.02 (5.28)	26.29 (32.88)
Use of tobacco prior to pregnancy (%)		36.6	0
Tobacco use during pregnancy (%)	1.7	6.7	0
Gestational Diabetes (%)		14.7	21.8
History of Asthma (%)	21.8	4 (0.6%)	9 (3.5%)
Number of Multiparous		380 (54.9%)	162 (62.3%)
Number Primiparous		312 (45.1%)	97 (37.3%)
Mean Annual Household income (>50K)	84%	80.4%	36.8%
Education level of mother (% with highschool)	92%	86.2%	99.2%

# Maternal Dietary Composition

	Child	Family	START-Canada
N	2605	625	243
Maternal total daily energy intake (kcal/ day)	1989 (729)	2183 (SD 744)	1926 (SD 683)
% total calories from Carbohydrates	49.2 (6.0)	55.9 (6.3)	58.1 (5.5)
% total calories from fat	33.2 (5.4)	29.2 (5.0)	28.9 (4.0)
% total calories from protein	17.5 (2.8)	16.8 (2.5)	16.1 (2.4)
Saturated fat grams/day	25.2 (11.6)	26.74 (11.6)	21.7 (9.3)
Trans fat grams/day	2.53 (1.33)	0.46 (0.52)	0.26 (0.33)
Polyunsaturated fat grams/day	15.6 (7.1)	9.67 (3.8)	11.78 (4.9)
P/S ratio	0.64 (0.18)	0.38 (0.12)	0.59 (0.22)
Glycemic Index of maternal diet	49.8 (3.2)	47.39 (4.1)	43.4 (5.5)
Fibre (grams/day)	24.21 (9.52)	19.16 (8.2)	23.52 (9.6)
Vegetarian	?	1.03%	40.3%

As of October 2012



# Objective 1

- To identify and study the dietary patterns of pregnant mothers which predict maternal health during pregnancy (i.e. weight gain, gestational diabetes, hypertension, post-partum weight retention), and newborn/infant/child health outcomes including adiposity, growth, metabolic traits (glucose, insulin, lipids, blood pressure), allergic disorders and asthma up to five years of age.

# Maternal Diet and Wheeze

- Osaka Maternal and Child Health Study
  - N=763 infants ages 16-24 mos
- Q4 v Q1 of “Western” maternal diet
  - High “white vegetables”, oil, salty seasonings, processed meat, eggs; low fruit
  - OR (adj) = 0.59 (0.35-0.98) of wheeze at 16-24 mos.

# Maternal Diet and Wheeze

- International Study of Asthma and Allergy in Childhood (ISAAC)
  - N=2441 children at 5 y
  - 181-item FFQ asking about diet habits in 8<sup>th</sup> month of pregnancy
- Low maternal consumption of leafy vegetables
  - OR (adj) = 1.55 (1.21, 1.98) of wheeze
- Low Malaceous fruits
  - (apricot, apples, peaches, plums, prunes)
  - OR (adj) = 1.45 (1.15, 1.84) of wheeze
- Low Chocolate
  - OR (adj) = 1.36 (1.09, 1.70) of wheeze
- High maternal consumption of Fruit and Berry Juices
  - OR (adj) = 1.40 (1.03, 1.90) of allergic rhinitis

# Example from FAMILY

- Recent analysis of FAMILY birth cohort (859 pregnant mothers from Hamilton Ontario), the polyunsaturated to saturated fat ratio in the maternal diet was very low (0.33), indicating a poor quality diet, high in saturated fat which was associated with larger body size of newborns

# Preliminary Power: Diet Effect

- **Outcome:** Adiposity OR of body fat >24% at 3 mo = 0.47
- **Diet:** High P:S vs Low P:S >0.70 vs <0.40

D: Anderson (2010) [Atlanta]; n=96 mother-infant pairs; High *trans* fat vs. low *trans* fat

OR	N	Power
0.50	5000	>0.99
0.75	5000	>0.99
0.77	5000	0.857
0.79	5000	0.783

## Prelim *CHILD* Data: Quartiles of Exposure (Energy Adjusted mean $\pm$ SD)

<b>Nutrient</b>	<b>Q1 N=645</b>	<b>Q2 N=645</b>	<b>Q3 N=646</b>	<b>Q4 N=645</b>
P:S Ratio	0.44 (0.06)	0.57 (0.03)	0.68 (0.04)	0.88 (0.13)
SFA (g)	18.5 (3.0)	23.3 (0.9)	26.2 (0.8)	31.4 (3.7)
SFA (%)	8.4 (1.1)	10.5 (0.4)	12.0 (0.4)	14.3 (1.4)
Carbohydrate (g)	203.6 (20.3)	233.1 (5.0)	249.2 (4.9)	277.9 (20.8)
Carbohydrate (%)	41.6 (3.5)	47.5 (1.1)	51.1 (1.1)	56.6 (3.3)

\*FFQ; Excluding those reporting <500 or >4500 kcal (n=23; 0.9%)

# Expected Events by end of Grant Award (2018)

Events	CHILD (n=3600)	FAMILY [n=800]	START (n=1000)	ABC (n=300)	Total
Gestational Diabetes	133	30	74	25	262
Hypertension in Pregnancy	90	20	25	24	159
Bottle Feeding from Birth	468	104	130	34	736
Skin Test + Allergy to Food	198	44	110	17	369
Skin Test + Allergy to Animal	1080	240	300	90	1710
Eczema	720	160	144	45	1069
Wheeze	720	160	167	60	1107
Asthma	360	80	50	17	761

# Preliminary Event Rate Assumptions: Pregnancy

Event	Assumed rate	START (South Asian)	ABC (Aboriginal)
Gestational Diabetes	3.7% CDA	7.4% CDA	8.4% Harris et al., 1997
Hypertension in pregnancy	2.5% Magee et al., 2009	2.5% Saeed et al., 2011 (India)	8.5% Heaman et al., 2006
Bottle feeding from birth	13% StatsCan (2011)	13% NHS Infant Surv. (UK 2005)	11.5% StatsCan (2011)



## Preliminary Event Rate Assumptions: Allergy/Asthma

Event	Assumed rate	CHILD	START (South Asian)	ABC (Aboriginal)
Asthma	10% CHILD estimate	10% @ 1yr	5% Kuehni et al, 2007 (UK)	5.7% Gao et al, 2008 (Can)
Food allergy	5.5% Health Canada (MD diagnosis)	5.6%	11% Kuehni et al, 2006 (suggests OR: 3.0-4.0)	5.5%
Pet allergy	30% Asthma and Allergy Foundation (US)		30%	30%
Eczema	20% Asthma and Allergy Foundaton	14.1%	14.4% Whithrow et al., 2010	15% Glasgow et al., 2003 (RR=0.75 vs AUS)
Wheeze	20% StatsCan	10.6% @ 1 yr	16.7% Kuehni et al., 2007	20% Gao et al., 2008

**I. Genotyping**

**II. Gene Expression**

**III. DNA Methylation**

•Human CoreExome BeadChip  
(*Illumina*)

•Human HT-12 BeadChip  
(*Illumina*)

•HumanMethylation450BeadChip (*Illumina*)

**Gene-Diet Interactions**

Genome-wide Gene Expression and Methylation  
N = 500 in Cord Blood DNA

Mothers DNA  
N=4,500

×

Maternal Diet



Maternal Outcomes

Cord Blood DNA  
N=4,500

×

Maternal Diet



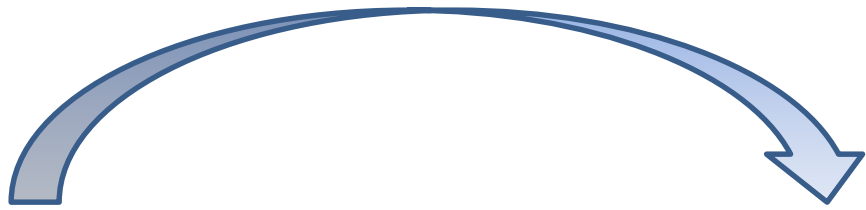
Infant Diet

Infant Outcomes

Maternal Diet



Targeted Methylation  
N=4,000




# Objective 1: Analytic Approach

- Step 1: Creation of nutrient profile for each cohort and common variables (some data cleaning will be required)
- Step 2: Bring nutrient and outcomes data together for group analysis
  - Food Patterns (Western vs Prudent vs Asian diet)
  - Specific Nutrients
    - Quartiles of Maternal PUFA
    - Saturated fat
    - Dietary glycemic index
    - Protein intake
    - Selected micronutrients
  - High power for these analyses

## Objective 2:

To investigate novel gene-diet interactions (maternal diet x maternal genotype, maternal diet x newborn genotype; newborn genotype x infant diet) and epigenetic marks of newborns, and relate these to infant/child outcomes including birth weight, adiposity, metabolic status, and atopy, allergic disorders, and asthma up to 5 years of age.

# Example: Gene Score Infant x Infant Diet Interaction

	Mostly Bottle fed (+)	Mostly Breast Fed (-)
Gene Score in Newborn (Quartile 4)		
Quartile 1,2,3		

# Design: Measurement, Outcomes, and Power

	CHILD	FAMILY	START	ABC
Maternal DNA	Yes	Yes	Yes	Yes
Cord Blood DNA	Yes	Yes	Yes	Yes
Cord Blood RNA	RNA	No	Yes	Yes
Infant 1 or 2 year DNA	Yes	No	Yes – Paper spot	Yes – Paper spot
Maternal FFQ	Yes	Yes	Yes	Yes
Infant Feeding Assessment				
-Breast feeding	Yes (56%	180 (29.95%)	26 (48.1%)	NA
-Introduction of Solids	exclusive to 3 mo)	563 (93.68%)	33 (61.1%)	NA
-Infant diet at 1 year	Yes	Yes	Yes	Yes
	Yes			

# Genotyping

- DNA Extraction (n=4,900)
- Plan: Use new Illumina Genome-wide gene chip  
500,000 SNPs (50% coding , 50% tagging)
- Select SNPs which have been robustly associated with:
  - - Birth weight
  - Adiposity
  - Cardio-metabolic traits
  - Allergy , atopy, asthma

# Gene Score

- Created for Mother and newborn separately
- Allele counting
- Example:
- A. Gene Score for body weight in mother x (prudent vs western diet) on newborn birthweight
- B. Newborn gene score x (breast vs bottle feeding) on weight at 1 year, allergy, asthma.

\*\* ? Adjust maternal GS for fetal GS and vice versa



# Power for Gene x Diet Interactions

- Used the most robust gene association we could find (from either candidate gene or GWAS studies)
- Used the best estimate of effect of diet on outcome
- At 80% power for the G x D, both main effects >98%
- Analyses conducted by Binod Neupane, PhD, SIGMA Lab

# Preliminary Power Curves: Gene x Diet Interactions

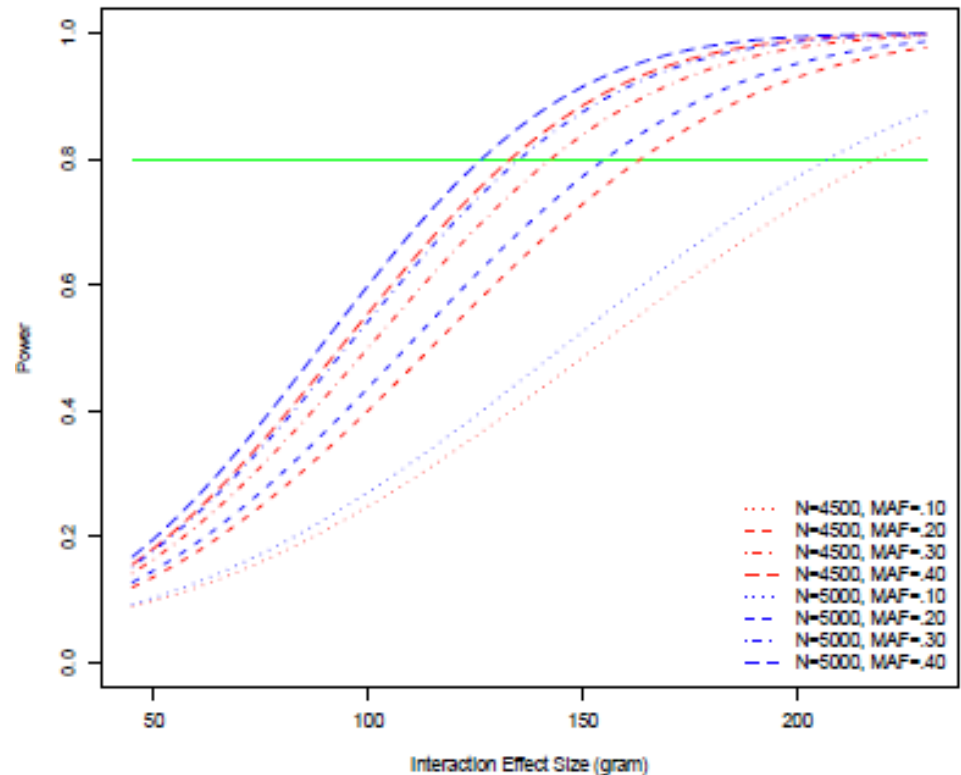
- Outcome: Adiposity weight gain from 0-1 yr
- Gene: FTO-rs9939609 (A allele) +87 g/year/allele
- Diet: Formula vs. Breast fed +119 g/yr

Power analysis for Adiposity (cont), N-Y BF (binary)

G: Vimalleswaran (2012), DioGENES,  
N=11091, XS; candidate gene (FTO)

D: Chu (2012), ProCoh (Toronto); N=246

125-225 g Interaction Effect/year



# Preliminary Power Curves: Gene x Diet Interactions

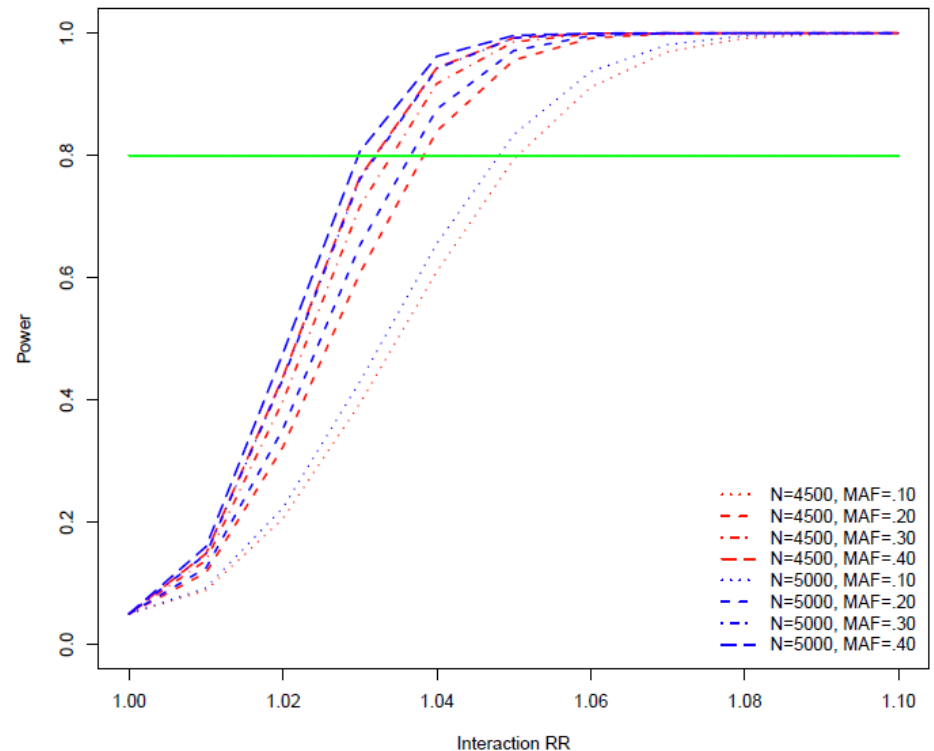
- Outcome: Adiposity >95<sup>th</sup> vs <85<sup>th</sup>
- Gene: Gene Score ( $\geq 13$  risk alleles vs  $\leq 4$ ) OR=1.17
- Diet: per month exclusive breastfeeding OR = 0.90

G: Elks (2010); ALSPAC; n= 7146; gene score

D: Scott (2012); ProCoh [NCNPAS (Aus)]; n= 2275;

1.03 – 1.05 RR Interaction effect/ mo  
0.95 - 0.97

Power analysis for Adiposity (bin), 1 less month BF (cont)

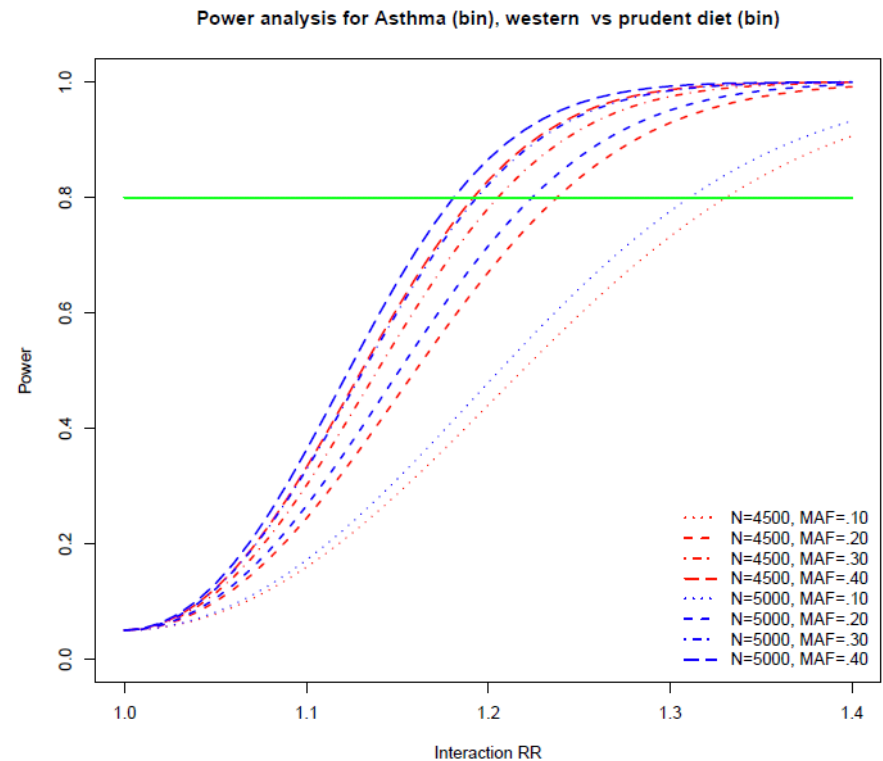


# Preliminary Power Curves: Gene x Diet Interactions

- Outcome: Asthma Yes vs. No
- Gene: *IL18R1* (A v G) OR=0.85
- Diet: Western v. Prudent OR= 0.60

G: Moffatt (2010); GWAS; n= 8730  
D: Miyake (2011); ProCoh [Osaka M/C health]; n=763

1.18 – 1.32 RR Interaction effect  
0.76 – 0.85



# Objective 2a: Analysis

- Maternal Genotype x Maternal Diet
- Outcomes:
  - - Gestational Diabetes
  - - Birthweight
  - - Food Allergy in Infant
  - - Eczema in Infant

# Objective 2b

- Maternal diet x fetal genotype
- Outcomes:
  - - Birthweight
  - - Glucose, insulin, lipids at birth

# Objective 2c

- Fetal genotype x infant diet
- Outcomes:
  - - Growth to one year
  - - Adiposity/kg weight to 1 year
  - - Allergy at 1 year
  - - Eczema at 1 year
  - - Asthma at 1, 3, 5 years

# Statistical Analysis

- How do we deal with ethnicity in the analysis?
- Assumptions:
  - Most SNPs demonstrated in GWAS in white Caucasians are polymorphic in S. Asians and Aboriginal people
  - Sometimes minor allele in WC is major allele in non-WC
  - Gene Score- uses the same risk allele in all groups
  - [Example Gene Score in Epidream]



# Statistical Analysis-2

- Adjust for “ethnic” in the Gene Score x diet interaction studies
- Look for effect modification by ethnic group
- 2) What about co-adjustment for Maternal and fetal genotypes?

# EpiGenetics

- Epigenetic analysis of newborn cord blood DNA using the Illumina 450K Infinium methylation assay to interrogate >450,000 methylation sites per sample at single-nucleotide resolution.
- Epigenetic patterns of infant will be compared by maternal dietary pattern, and by comparing high and low consumers of PUFA/saturated fat ratio and glycemic load AND
- Related to outcomes: birth weight, adiposity, glucose, allergy, asthma

# Maternal Carbohydrate and Epigenetic Profile

- 15 randomly selected umbilical cords from Princess Anne Hospital study participants
  - Lower maternal carbohydrate in early pregnancy associated with higher RXRA chr9:136355885+ methylation

