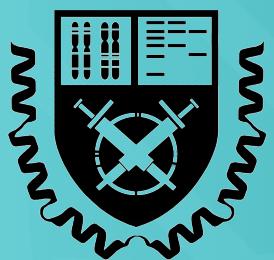


# Mosaic Genome-wide Paternal Uniparental Disomy

A Molecular Analysis of the 19<sup>th</sup> Case

McMaster  
University



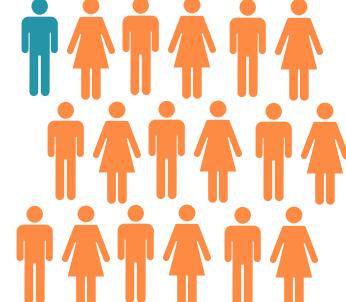
Bushra Haque

# Introduction



## Over 300 million individuals

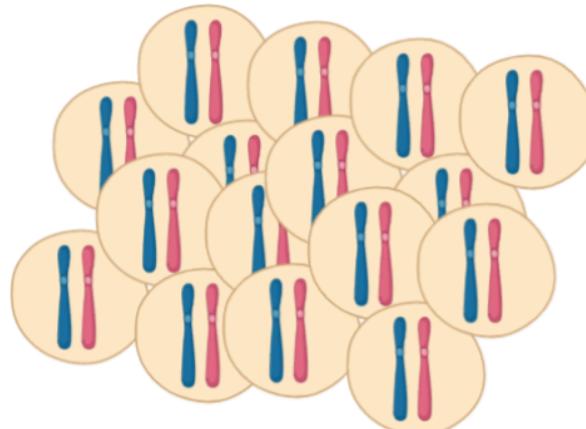
are living with one or more of the 6,800 identified rare diseases

- 72% are genetic 
- 1 in 2,000 individuals affected 
- Limited amounts of literature 

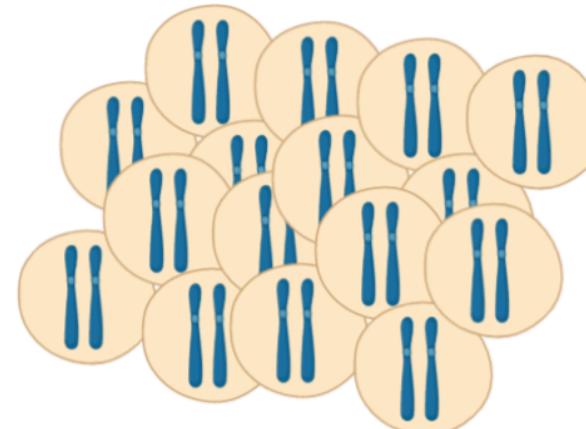
# Mosaic GWpUPD

## Background

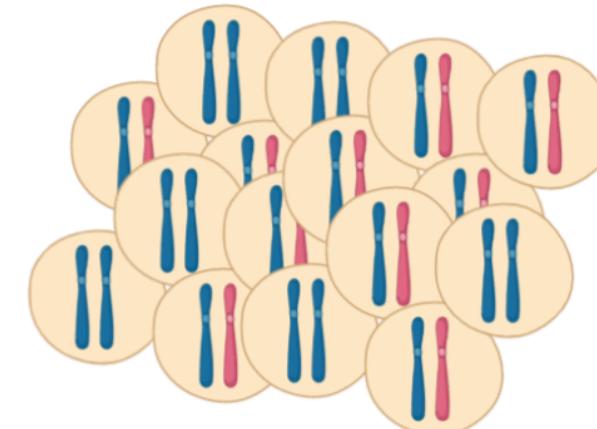
Images created in BioRender



Normal



Non-mosaic  
Paternal UPD



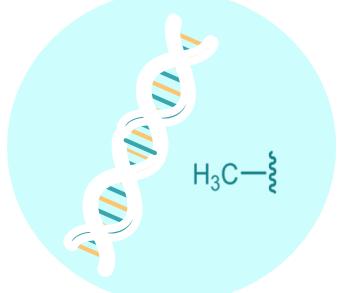
Mosaic Paternal  
UPD

- Somatic mosaicism of two cell lines
  - a) Cells with paternal & maternal chromosomes
  - b) Cells with two paternal copies of all chromosomes

# Genomic Imprinting

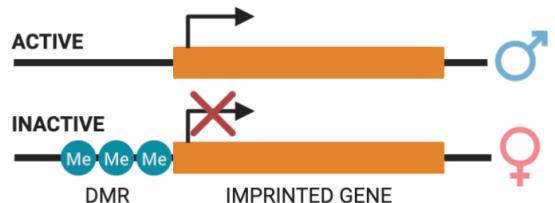
monoallelic gene expression by parent of origin-specific manner

## Epigenetic Modifications



- DNA methylation designates the parent of origin
- Renders one of the two copies of the genes active

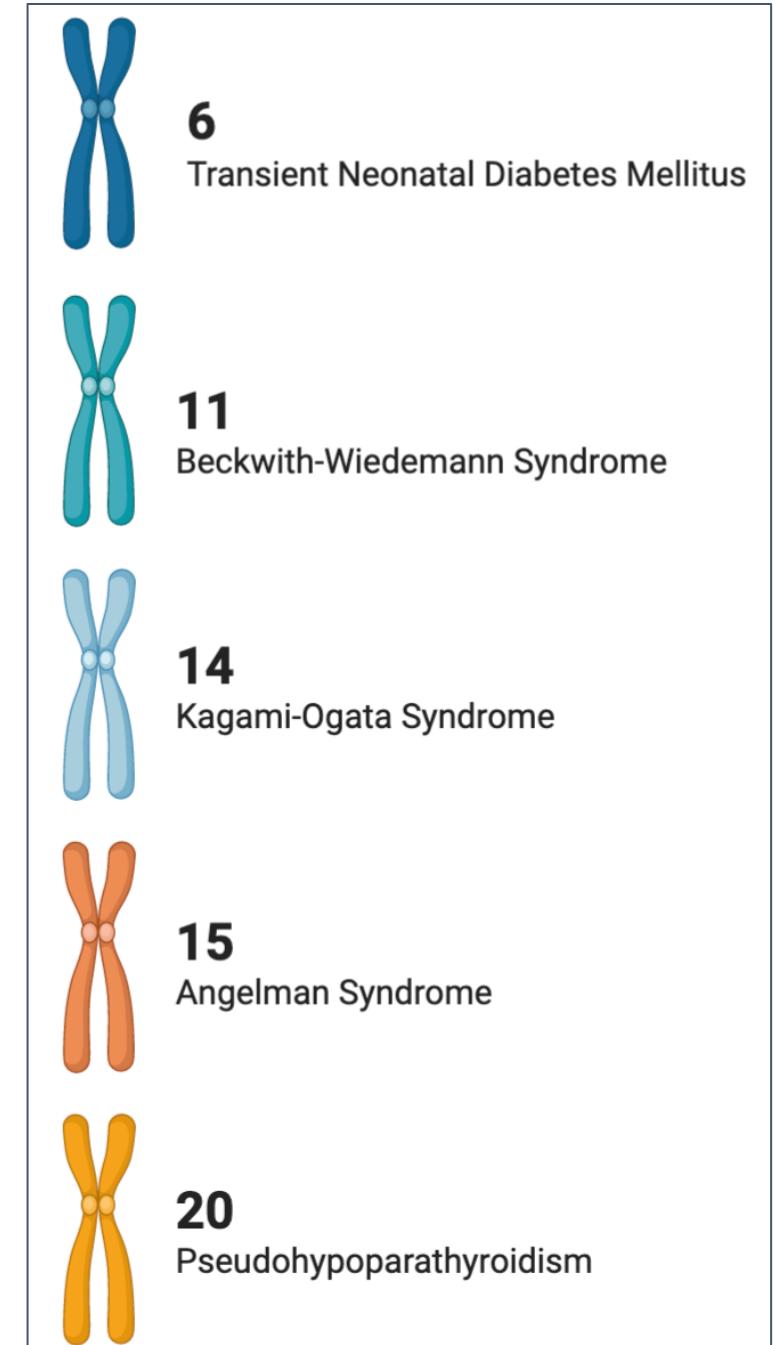
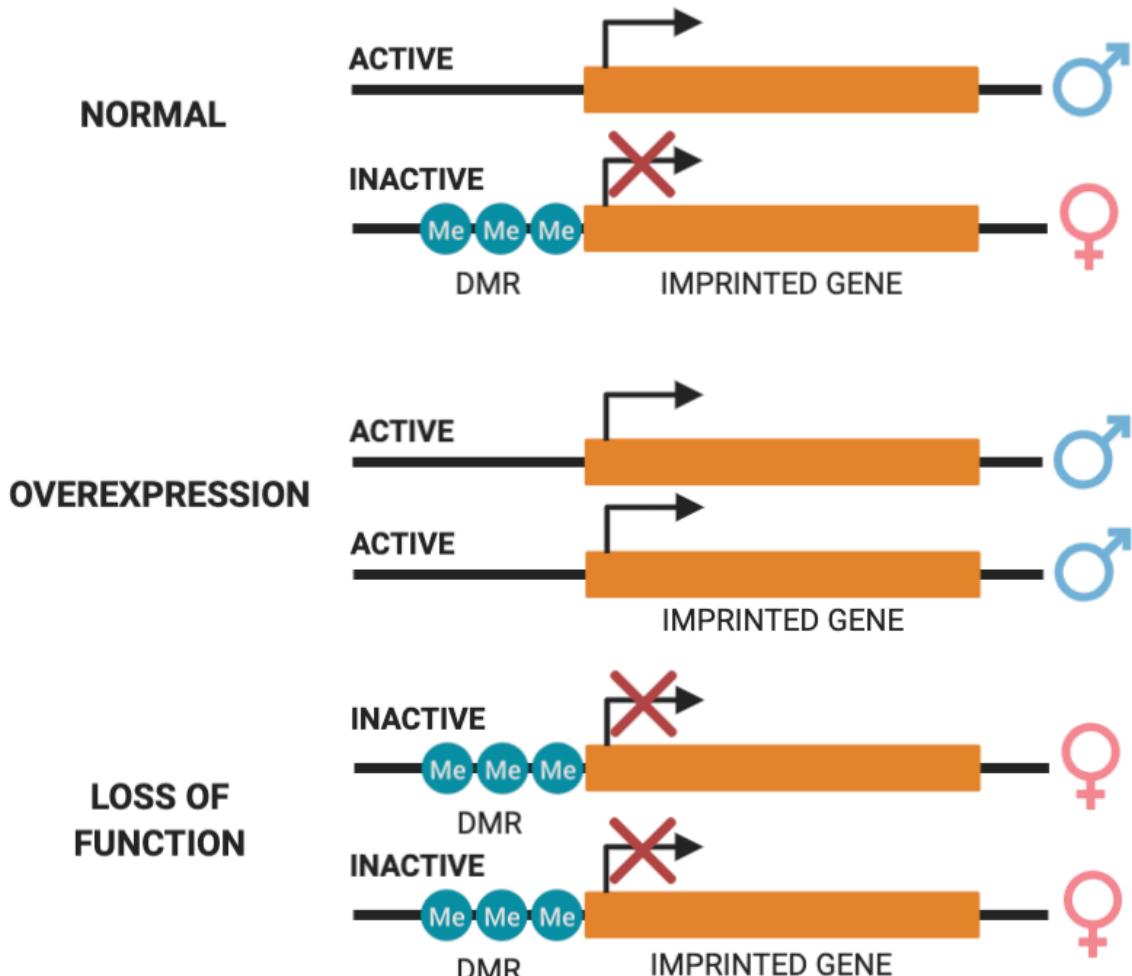
## Imprinted Genes

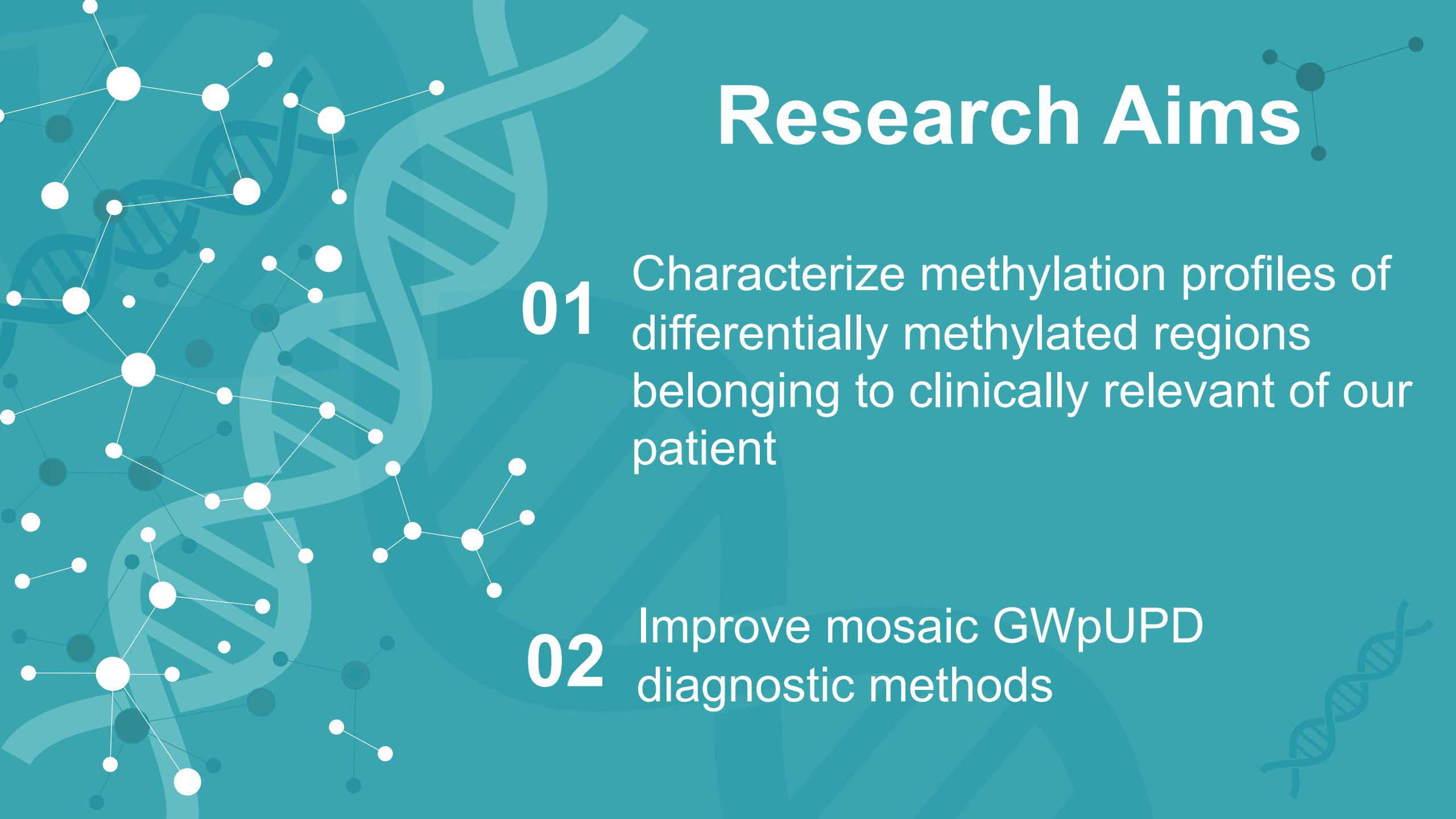


- Clustered in domains including:
  - Paternally/maternally expressed genes
  - Protein-coding genes
  - Non-coding RNAs
- Controlled by imprinted control regions characterized by differentially methylated regions

# Imprinting Disorders

Consequences of Uniparental Disomy





# Research Aims

01

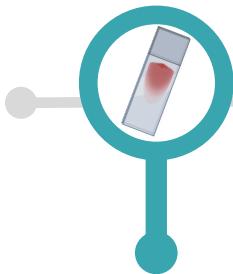
Characterize methylation profiles of differentially methylated regions belonging to clinically relevant of our patient

02

Improve mosaic GWpUPD diagnostic methods

# Methods

## Sample Collection



Genomic DNA isolated from whole blood, fibroblasts, buccal, & two placenta samples

Aneufast Multiplex QF-PCR kit used to amplify and quantify DNA



## QF-PCR

## Illumina Infinium MethylationEPIC



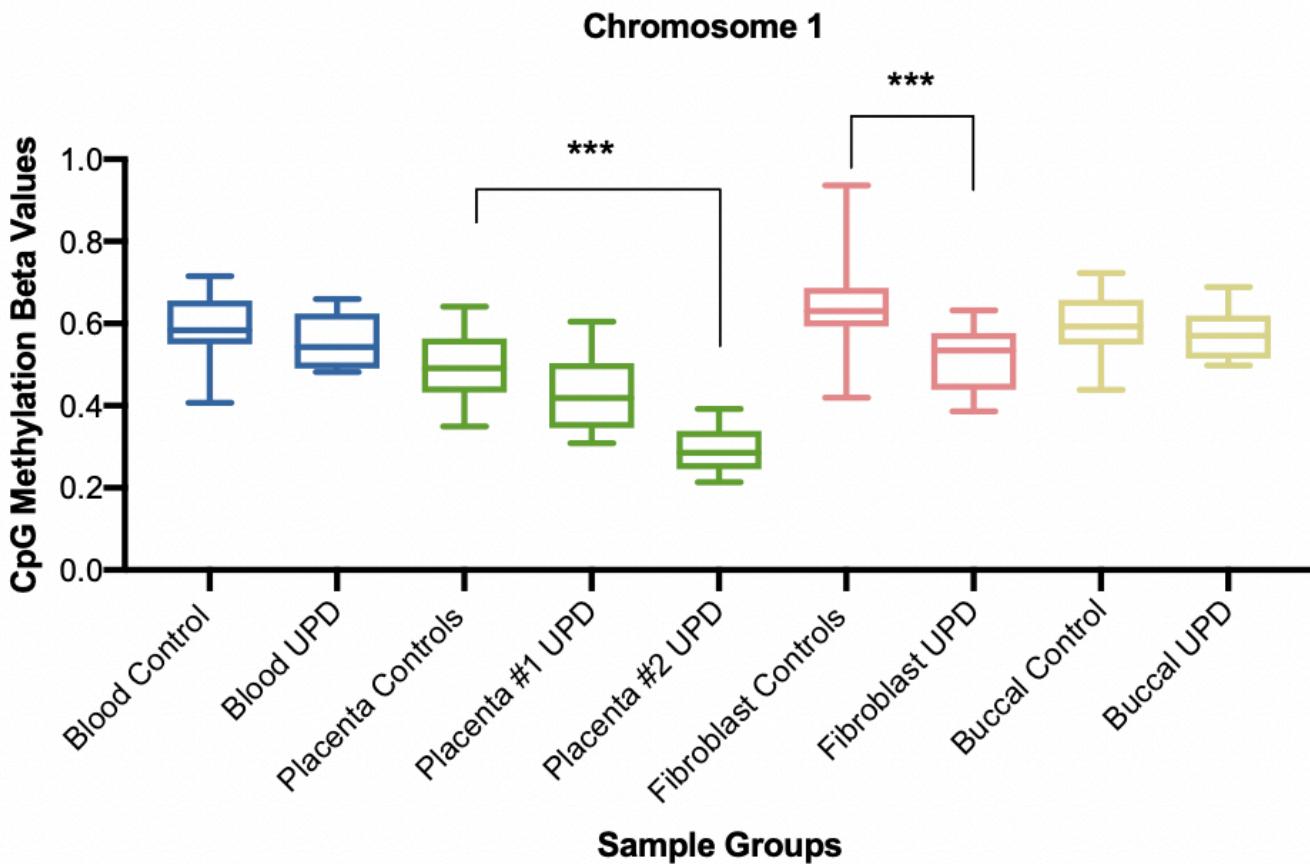
Microarray-based genome-wide DNA methylation analysis

Mann-Whitney U analysis



## Statistical Analysis

## CpG Methylation Beta Values of Known Maternal Imprinted DMRs on Chromosomes 1, 6, 7, 11, 15, 19 & 20 for Various Tissue Samples of UPD Patients and Control Groups



- Mann-Whitney U tests report significant differences in DNA methylation between **GWpUPD** patients and **control** samples in chromosomes **1, 6, 7, 19 and 20**
- The **placenta #2** and **fibroblast** cell lines exhibit mosaic GWpUPD

# Imprinted Genes with Lower CpG Methylation Beta Values



**6**

Transient Neonatal Diabetes Mellitus → *PLAGL1*



**11**

Beckwith-Wiedemann Syndrome → *KCNQ1*



**14**

Kagami-Ogata Syndrome

→ Lack of clinically  
relevant CpG sites  
within BeadChip array



**15**

Angelman Syndrome

→ *SNURF*



**20**

Pseudohypoparathyroidism

→ *GNAS*



**1**

No known phenotype

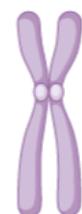
→ *DIRAS3*



**7**

No known phenotype

→ *ZIM2, PEG3*



**19**

No known phenotype

→ *GRB10, MEST, PEG10*

# Percent Mosaicism

Percentage of cells with paternal UPD in each sample type

## Results

- No mosaicism in blood sample (~1%)
- High mosaicism in fibroblast and placenta #1 samples (~35%)
- No maternal allele contribution in placenta #2 sample

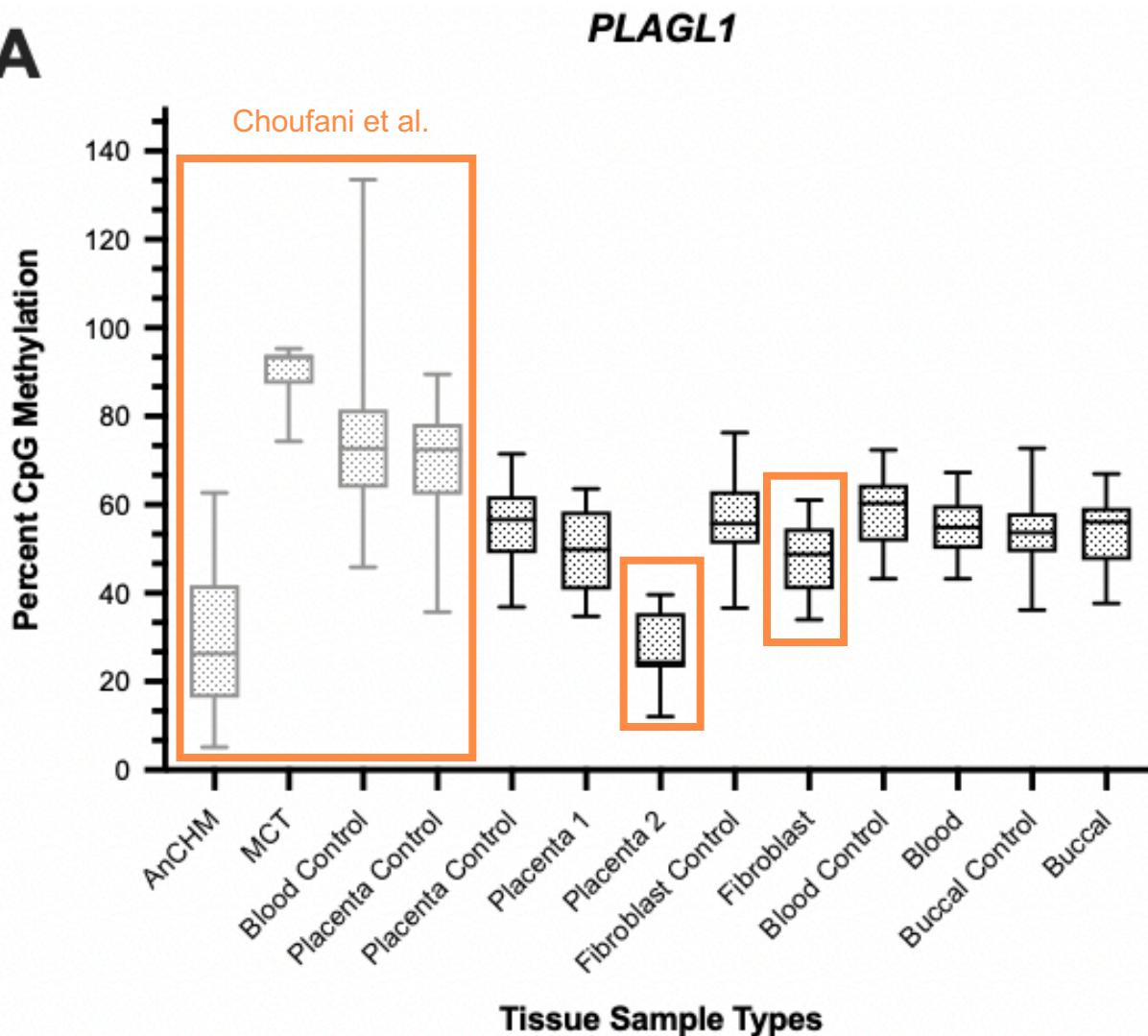


## Results

- No mosaicism in blood and buccal samples (<7%)
- High mosaicism in placenta #2 sample (~48%)
- Moderate mosaicism in fibroblast and placenta #1 samples (~14%)

# Variability of DNA Methylation

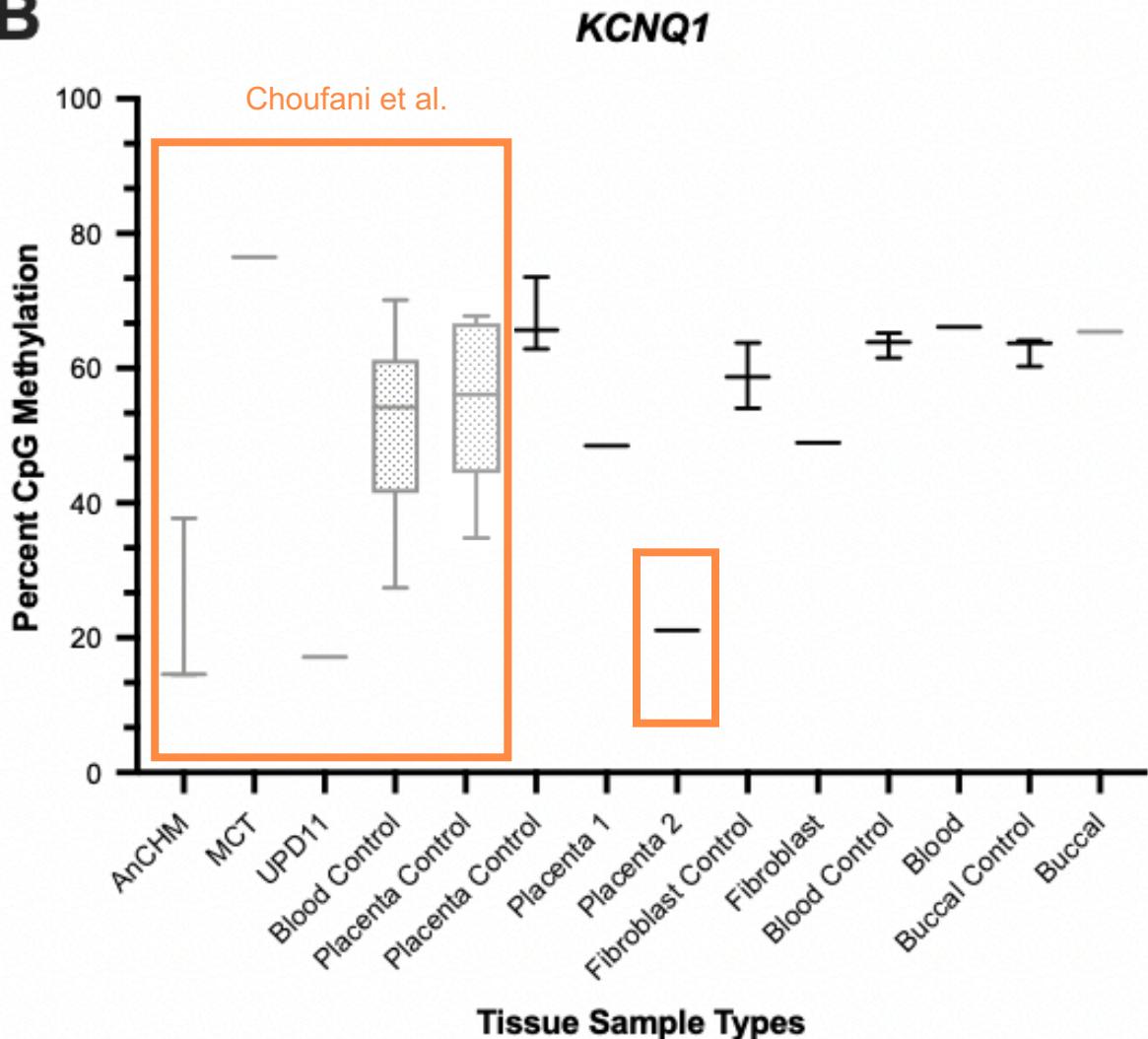
A



- Non-mosaic tissue types:
  - **AnCHM** = hydatidiform mole ( $\beta=0$ )
  - **MCT** = mature cystic teratoma ( $\beta=1$ )
- Placenta #2 and fibroblast samples exhibit **low methylation levels (<62%)**

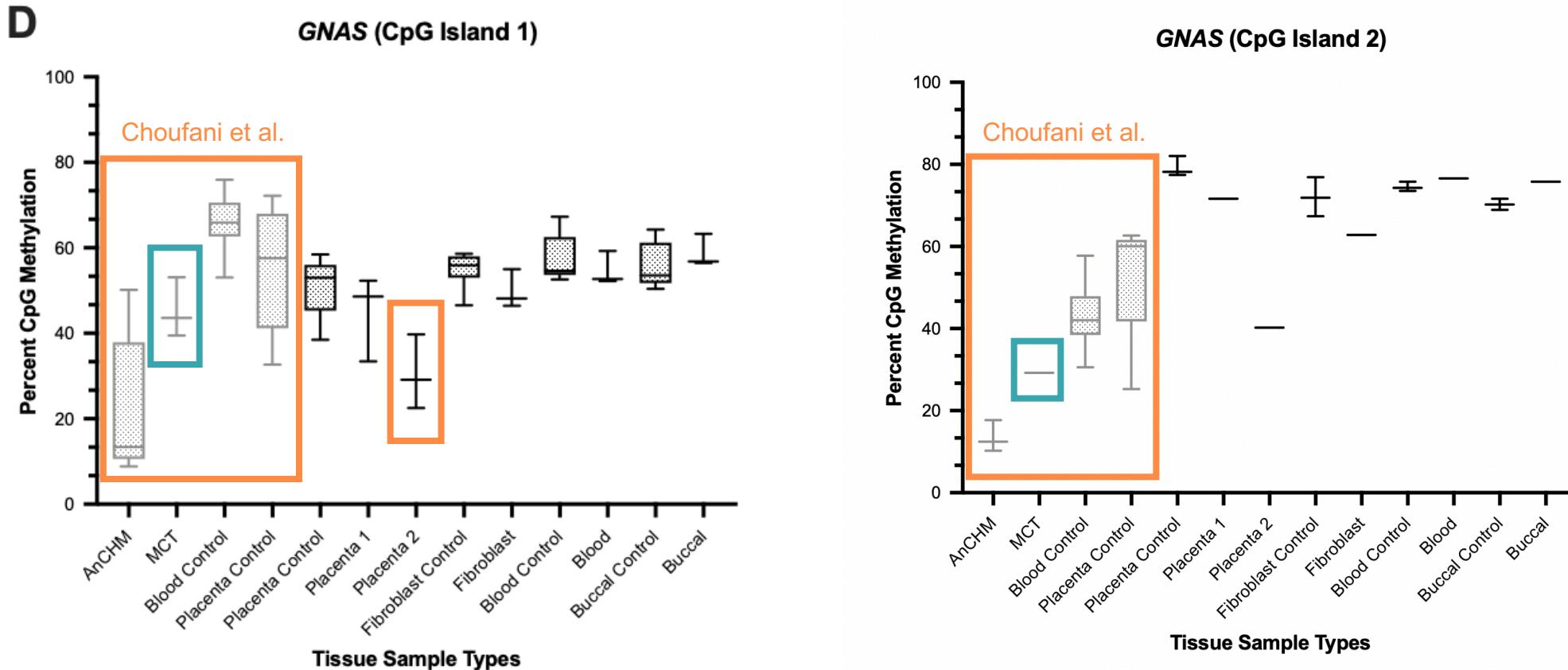
# Variability of DNA Methylation

B



- Non-mosaic tissue types:
  - **AnCHM** = hydatidiform mole ( $\beta=0$ )
  - **MCT** = mature cystic teratoma ( $\beta=1$ )
  - **UPD11** = lymphoblastoid cells of UPD11 patient
- Placenta #2 sample exhibits **low methylation** levels (<39%) and resembles UPD11 patient

# Variability of DNA Methylation



- Placenta #2 sample exhibits **low methylation** levels (<50%) in CpG Island 1
- **NOTE:** MCT sample also exhibits low methylation values
  - *Why?*

# Diagnostic Testing

Illumina Infinium  
MethylationEPIC  
BeadChip Array



CAPTURES VARIABILITY IN  
METHYLATION



PERFORMS QUANTITATIVE  
ANALYSIS



ANALYZES 850,000 CpG  
SITES

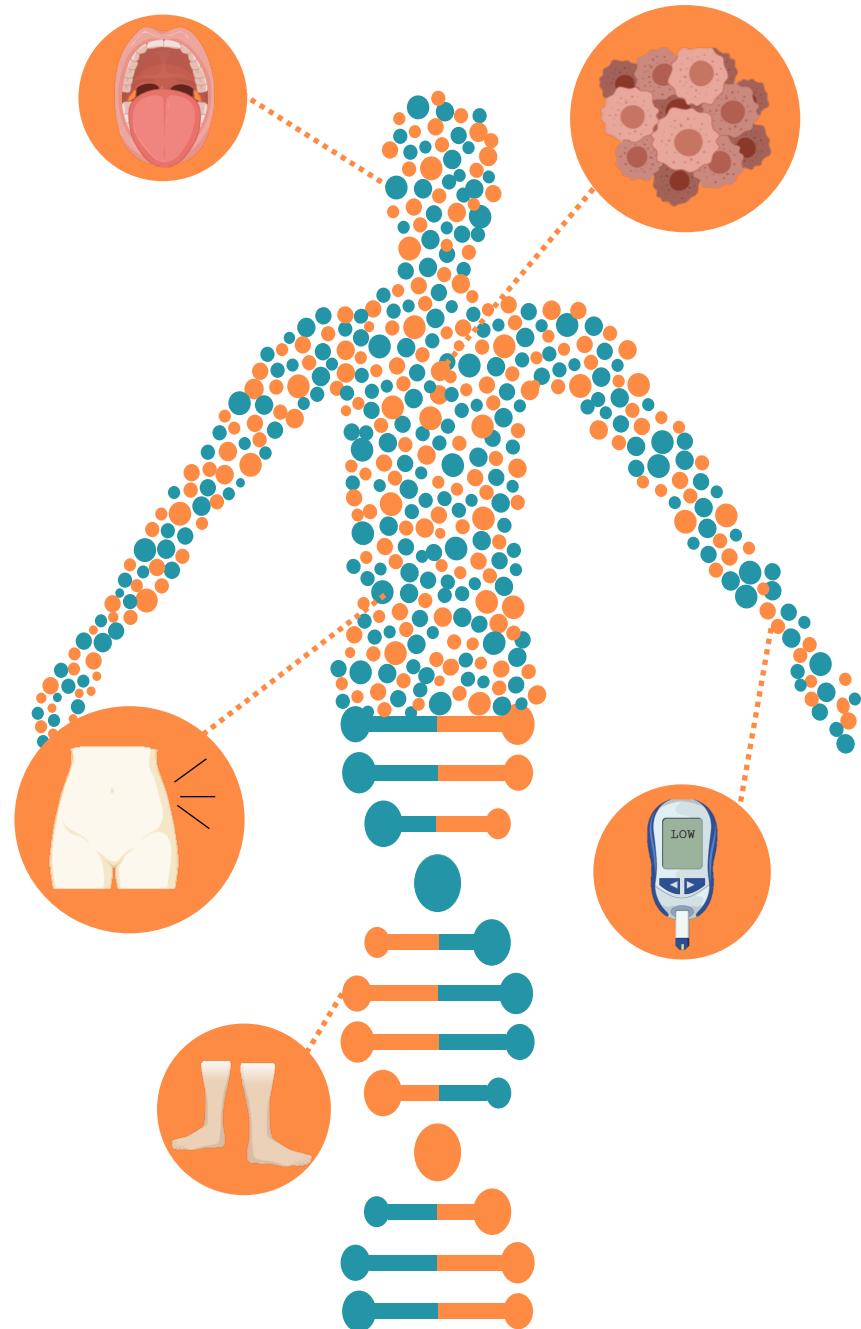


NOVEL PLATFORM FOR  
MOLECULAR DIAGNOSIS

# Phenotypes

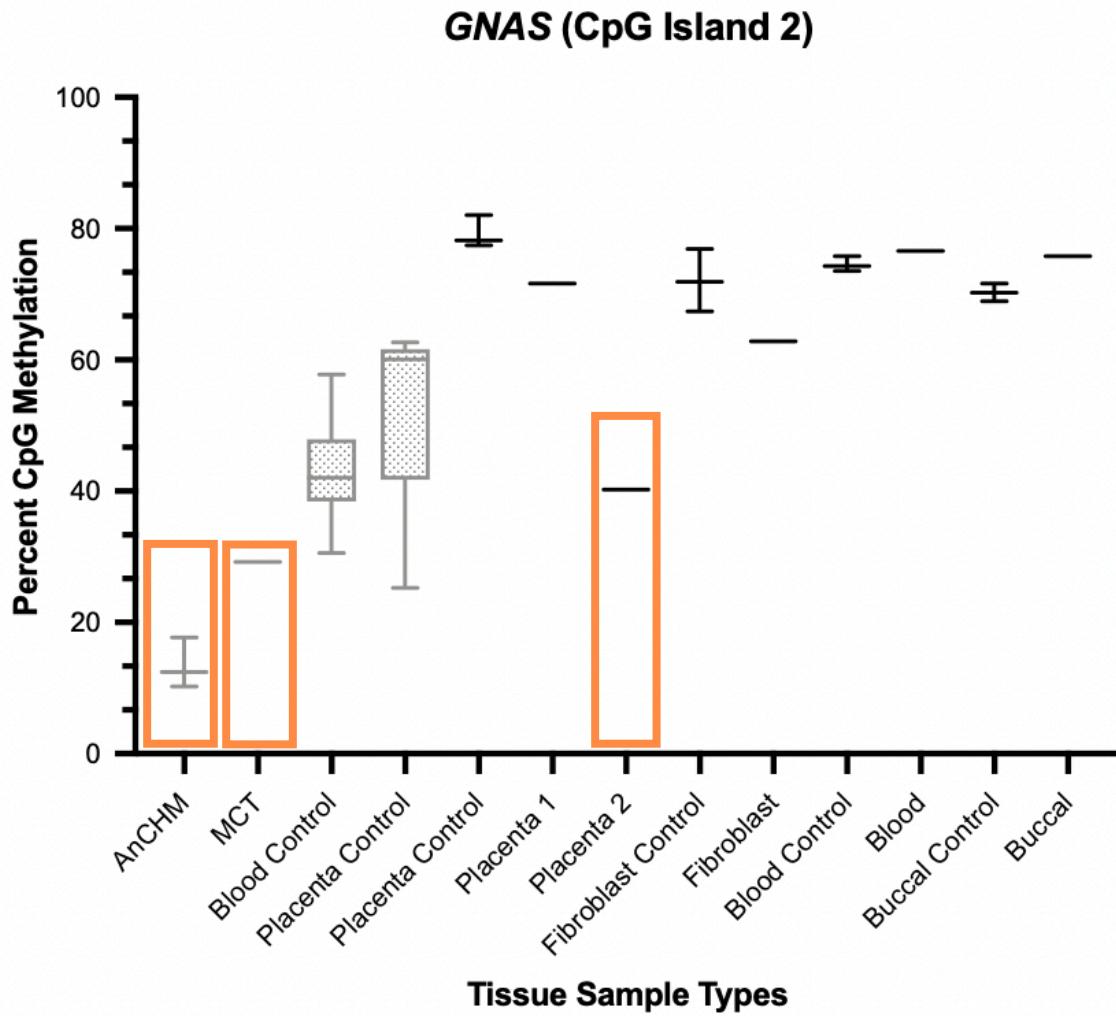
## UPD11 Phenotype Dominance

- Features of Beckwith- Wiedemann Syndrome are most predominant
  - Tissue-specific effects of imprinted genes
  - Mosaicism threshold
- Increased tumour risk
  - Inactivation of tumour-suppressor genes
  - Activation of oncogenes
  - Loss of *H19* expression



# Future Studies

## Exploring Biological Variation



### GAMETOGENESIS METHYLATION

#### Simple Model

- AnCHM – 0%
- Controls – 50%
- MCT – 100%

### COMPLEX INTERACTIONS/ POSTZYGOTIC METHYLATION

#### Complex Model

# Conclusion



Successfully characterized of mosaic GWpUPD in specific tissue samples belonging to our patient using the Illumina Infinium MethylationEPIC BeadChip kit

## INVESTIGATION OF IMPRINTED DMRs

Analyzed of CpG methylation beta values from BeadChip microarray

01



## NOVEL RESEARCH

## PERCENT MOSAICISM

Determined tissues exhibiting highest mosaicism from microarray and QF-PCR data

02



## TUMOUR SURVEILLANCE

## BEADCHIP MICROARRAY

Captured biological variation of DNA methylation

03



## CONTINUOUS MONITORING

# Acknowledgements

Dr. Daria Grafodatskaya, Assistant Professor

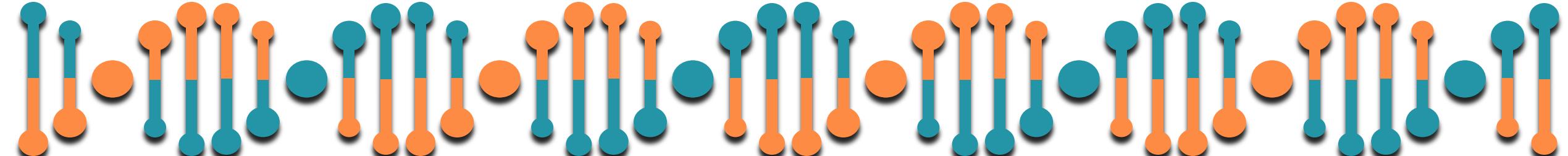
Dr. Darci Butcher, Clinical Molecular Genetics Trainee

Dr. Andrew McArthur, Associate Professor

Dr. Ben Bolker & BioData Lunch Team

Dr. Felicia Vulcu, Assistant Professor

Biochemistry Peers





**Thank You**  
**QUESTIONS?**

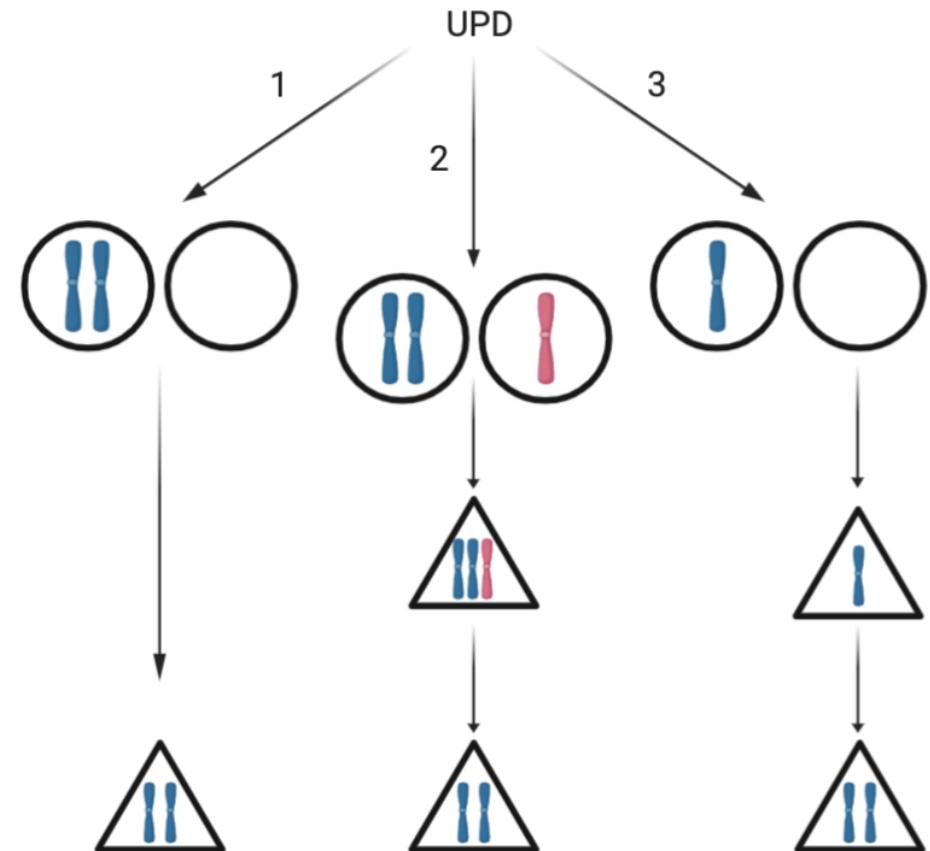
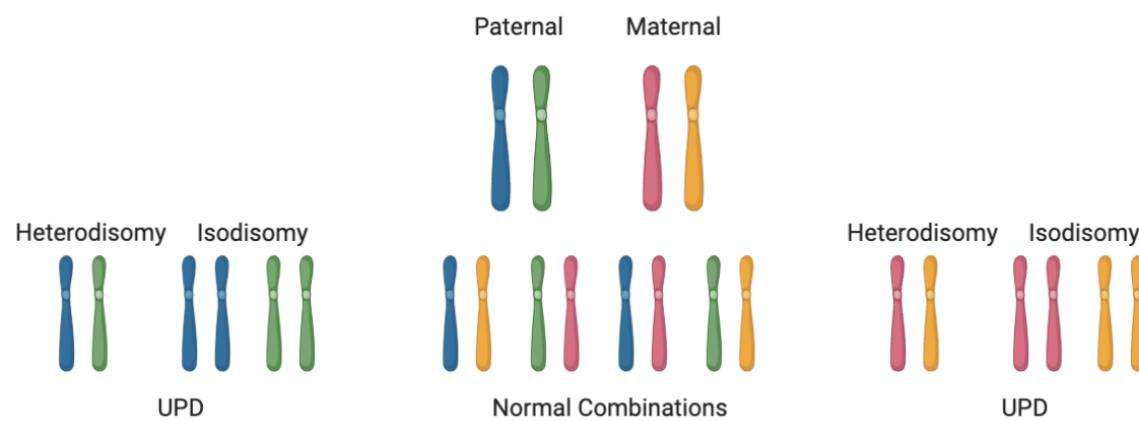
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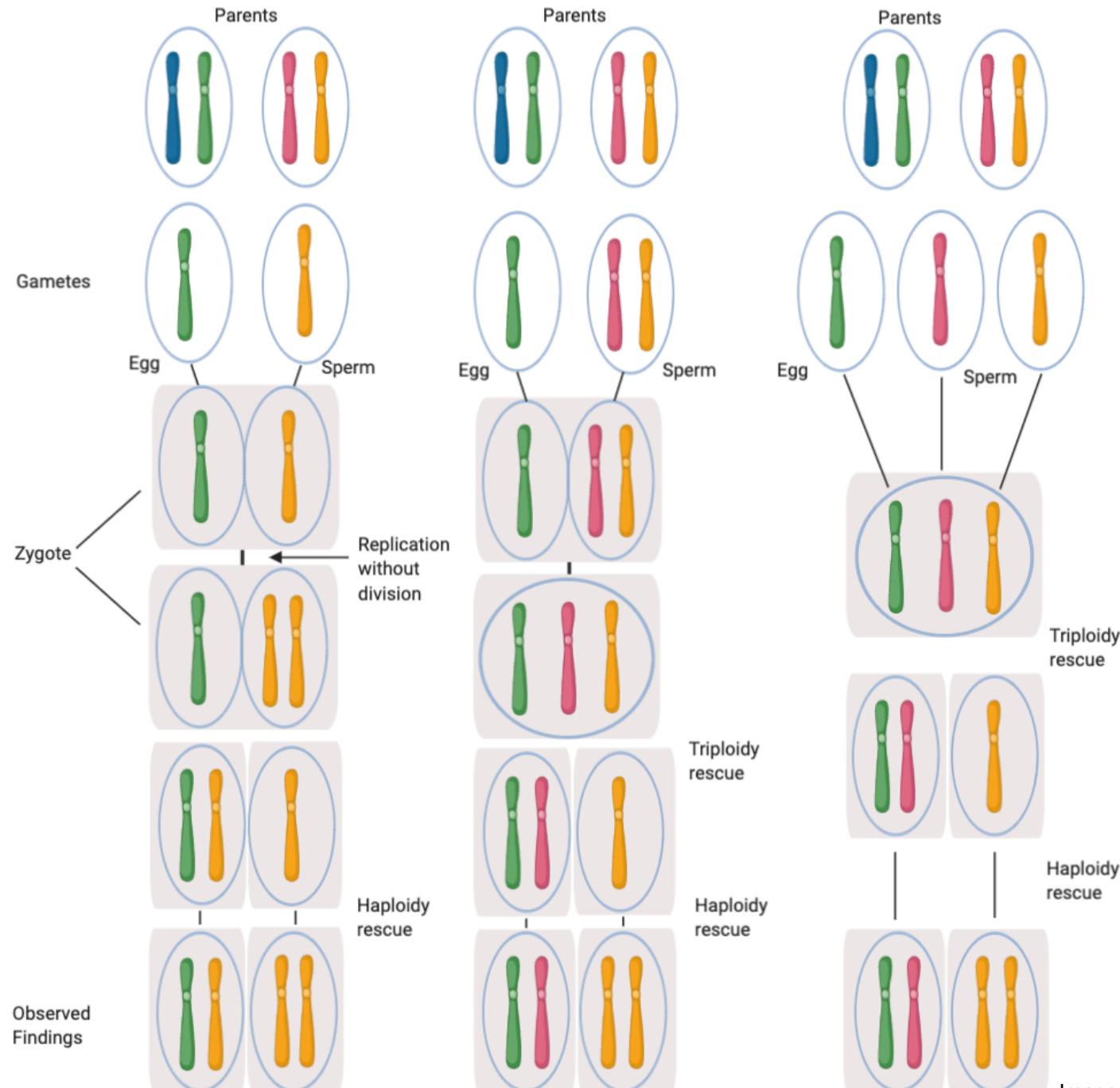
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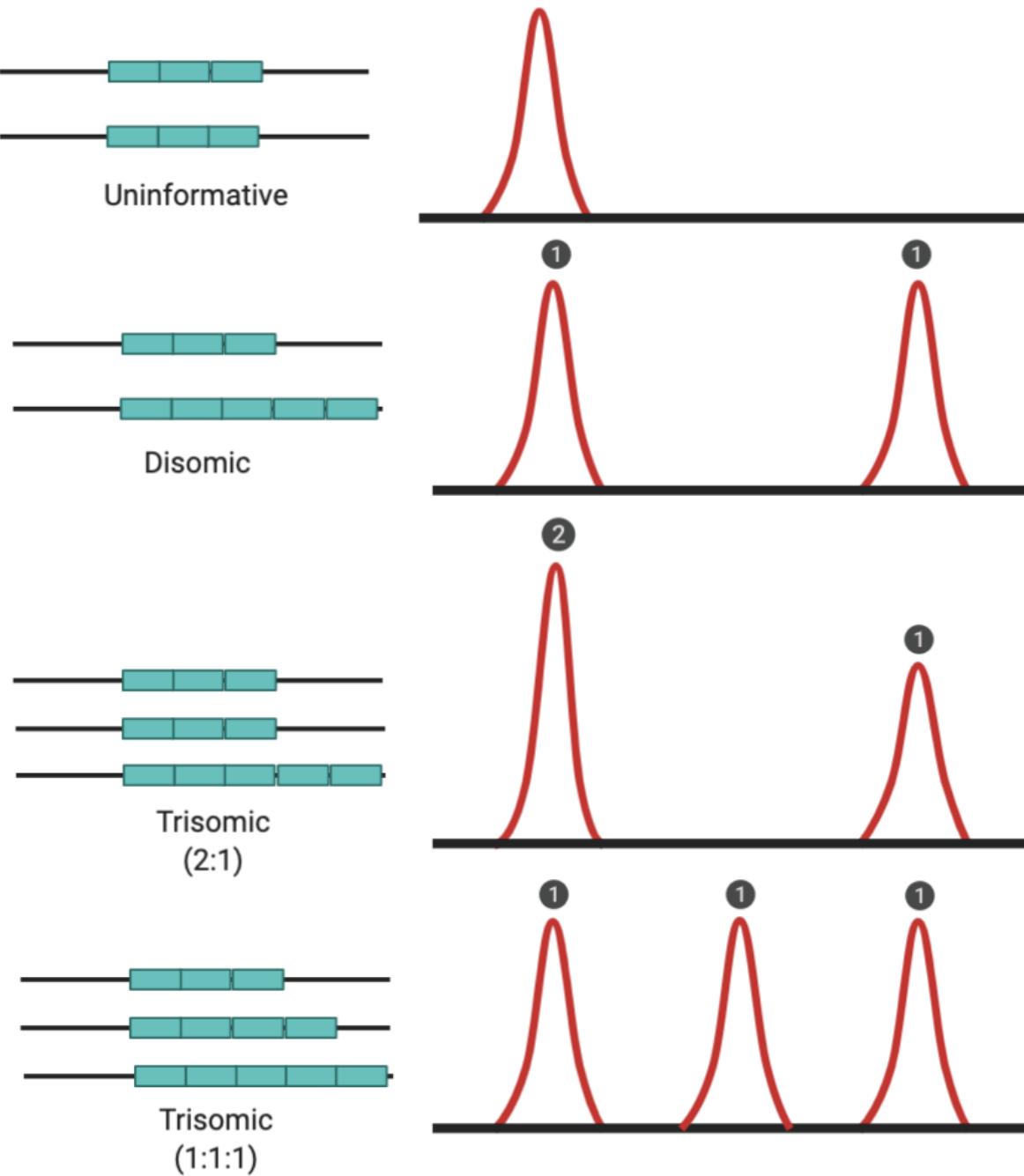
# Uniparental Disomy (UPD)



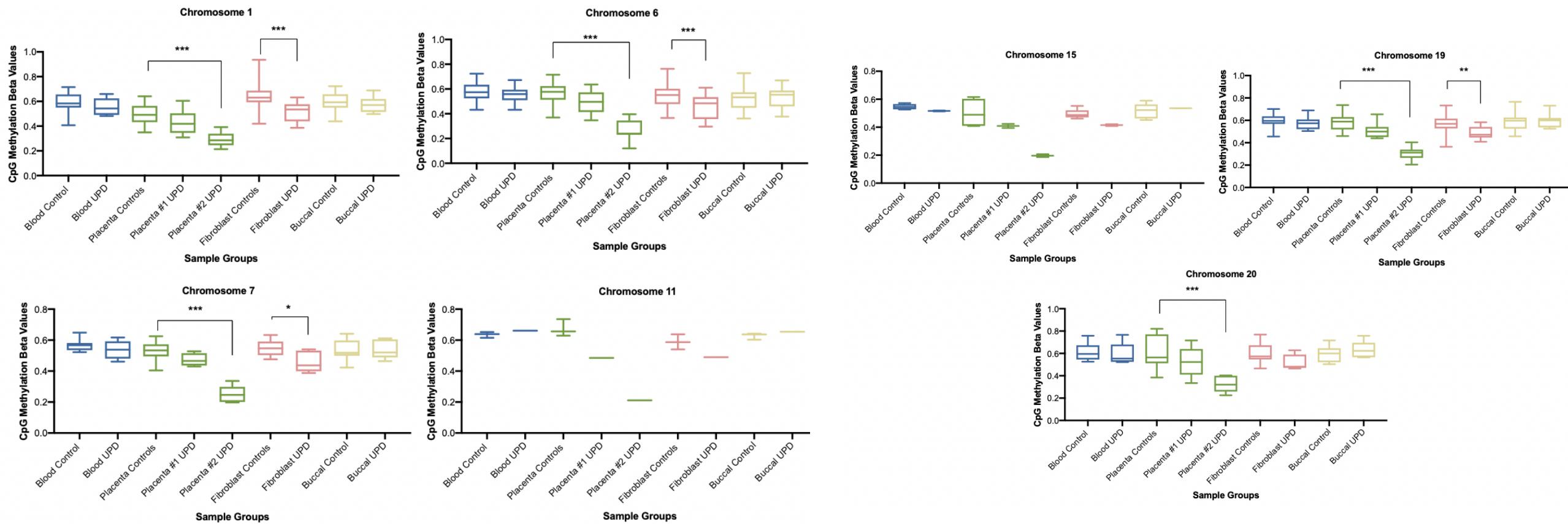
# Mosaic GWpUPD

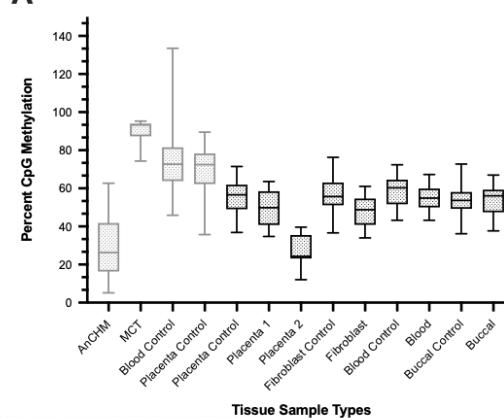
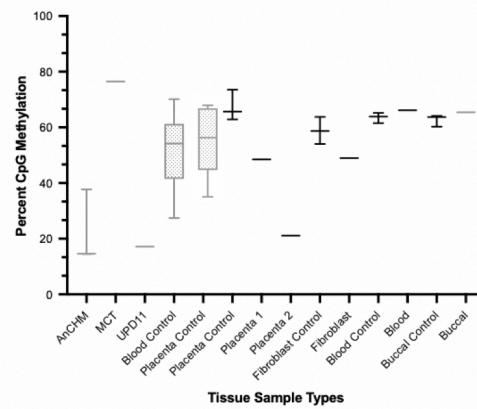
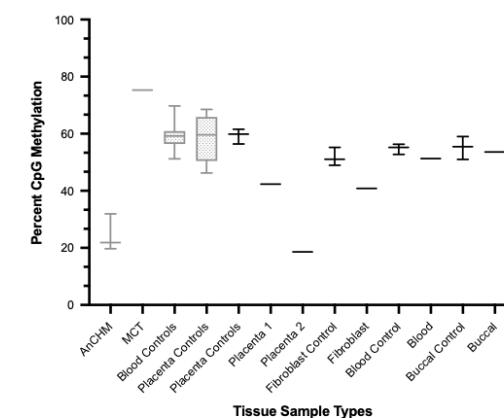
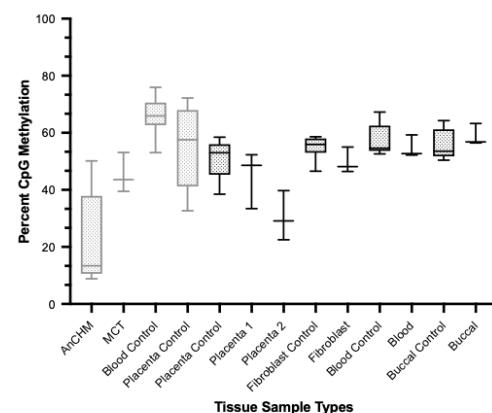
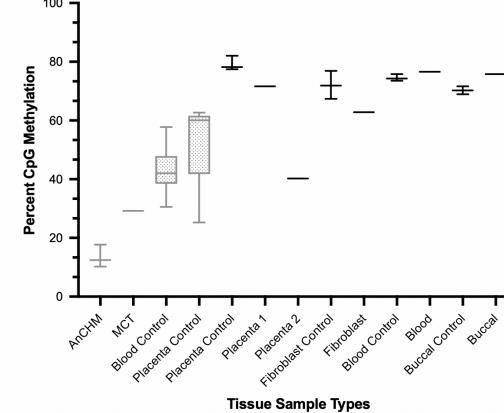


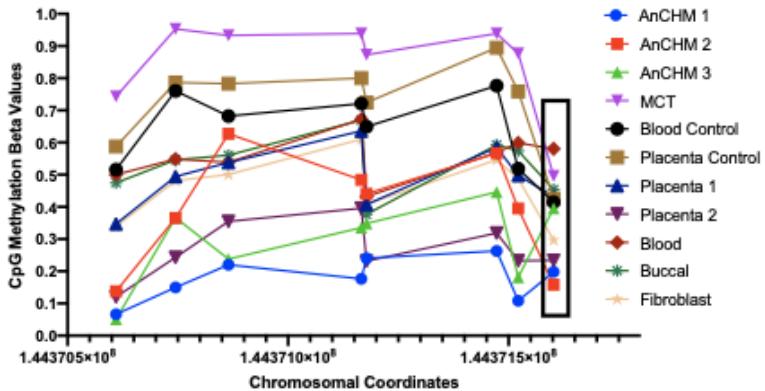
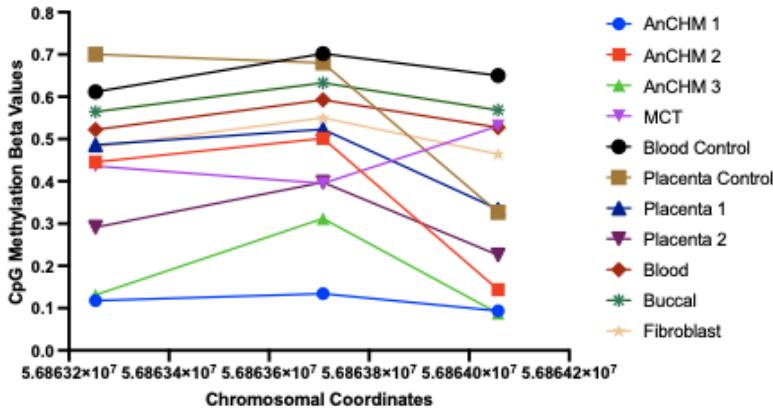
# QF-PCR Data



# CpG Methylation Beta Values of Known Maternal Imprinted DMRs on Chromosomes 1, 6, 7, 11, 15, 19 & 20 for Various Tissue Samples of UPD Patients and Control Groups



**A***PLAGL1***B***KCNQ1***C***SNURF***D***GNAS (CpG Island 1)***E***GNAS (CpG Island 2)*

**A**Location and Methylation Levels of *PLAGL1* DMRs**B**Location and Methylation Levels of *GNAS* DMRs in CpG Island 1**C**Location and Methylation Levels of *GNAS* DMRs in CpG Island 2