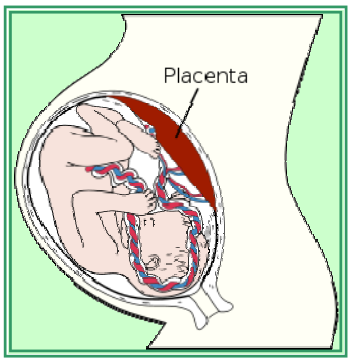
Newsletter Summer 2013

Robinson Lab *Reproductive Genetics Research at UBC and the Child & Family Research Institute*

THANK YOU!

First of all we would like to thank everyone who has generously volunteered to participate in our research studies! You make our research possible and play an important role in furthering our understanding of epigenetics and reproductive health. This newsletter is to share with you what you have helped achieve.

We focus most of our research on the **placenta**, an organ that is essential to provide nutrition to and regulate growth of the baby. A placenta that does not function well can lead to pregnancy complications. The placenta absorbs important nutrients, antibodies, and oxygen from the mother's blood and transports these to the growing baby. In return, waste products are transferred back to the mother's blood where they are exported from her body. In addition, the placenta produces important hormones and protects the baby from the mother's immune system that may recognize the fetus as a foreign intruder.



http://en.wikipedia.org/wiki/File:Placenta.svg

Keeping the placenta working well depends on specific genes being turned on and off in the right cells and at the right time of development. We are particularly interested in understanding how genes and the environment interact to influence gene expression in the placenta.

Epigenetics is the study of chemical changes to DNA, or to proteins that the DNA interacts with, that influence gene expression without changing the DNA code itself. These changes can be influenced by the environment and have long term effects. We are very interested in understanding how environmental factors influence epigenetic changes that relate to reproduction and development. **DNA methylation** is one kind of epigenetic change we are especially interested in.

Our Main Areas of Interest

- Preeclampsia and intrauterine growth restriction (IUGR)
- Recurrent Miscarriage
- Neural tube defects
- Chromosomal abnormalities

We are trying to learn as much as we can about the causes and features of these conditions and hope to move towards less invasive prenatal testing.

Current Lab Members

- Wendy P. Robinson, PhD. Professor, Dept. of Medical Genetics UBC.
- Maria S. Peñaherrera, PhD, Research scientist (Epigenetic studies of imprinting disorders and of neural tube defects)
- Courtney Hanna, PhD (recurrent miscarriage and infertility)
- Irina Manokhina, PhD, postdoctoral fellow (non-invasive prenatal diagnosis)
- Kirsten Hogg, PhD, postdoctoral fellow (stress and the placenta)
- Magda Price, PhD student (methylation and congenital diseases of the spinal cord)
- John Blair, MSc student (preeclampsia and DNA methylation)
- Samantha Wilson, MSc student (preeclampsia and DNA methylation)
- Tanjot Singh, Biology Honors student
- Campbell Drohan, Work Study student
- Ruby Jiang- Research associate/tech
- Kristal Louie, Msc - Research coordinator
- Joanna Mendell, BSc, MPH student – Research coordinator

To find out more about our research studies please contact us**: mosaic@cw.bc.ca** or see our **website:**
www.robinsonresearch.ca

Newsletter Summer 2013

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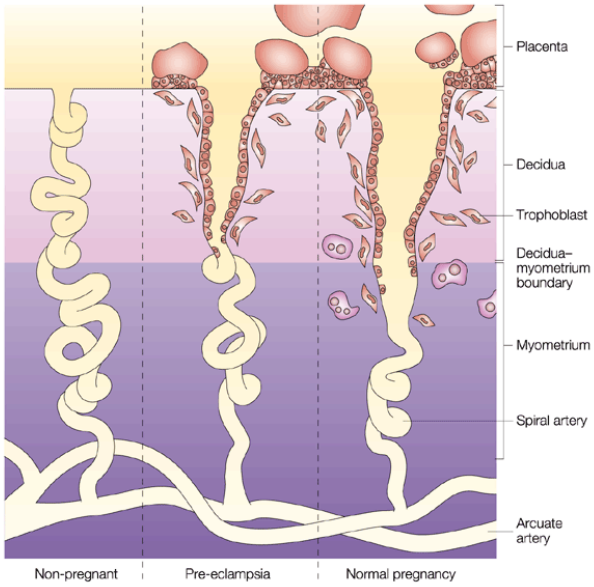
Preeclampsia and Intrauterine Growth Restriction

Babies born prematurely and underweight, often caused by poor placental function, are at risk of many complications in early life. **Preeclampsia** (hypertension plus protein in the urine) is an example of poor placental function where both the mother and baby can become very ill. If left untreated preeclampsia can develop into eclampsia and seizures occur. Early identification of at-risk women allows for more careful monitoring of the disease to save lives. Early-onset preeclampsia (EOPET), which occurs prior to 34 weeks gestation and is often more severe, is thought to be due to poor invasion of the placenta into the uterus. When this happens, insufficient blood flow to the placenta causes oxygen shortage in placental cells, and can lead to poor placental health. Our ultimate goal is to identify some of the early placental changes associated with EOPET that could be used to predict complications before they occur.



Leptin and Preeclampsia

Increased levels of a hormone called leptin are found in the blood of women with preeclampsia. Leptin, which is produced by our fat cells and regulates appetite, is also made in the placenta and is very important for cell growth, nutrient supply to the fetus, and regulation of hormones. Kirsten Hogg, a postdoctoral fellow in our lab, is interested in the relationship between stress, hormones, and the placenta. Kirsten's recent publication looks at how DNA methylation changes in the leptin gene could explain differences in leptin expression in preeclampsia. In the placenta DNA methylation was lower at the leptin gene in patients with early onset preeclampsia but not changed in late onset preeclampsia. Also, DNA methylation did not differ in placentas of babies that were underweight when preeclampsia was absent in the mother. These results help to explain the corresponding increase in leptin in maternal blood in preeclampsia. Many other changes in gene DNA methylation are expected to occur in preeclampsia and are important to study to better understand this placental condition and help to develop targeted preventative interventions.

Hogg K, Blair JD, von Dadelszen P, Robinson WP. Hypomethylation of the LEP gene in placenta and eleveated maternal leptin concentration in early onset pre-eclampsia. Mol Cell Endocrinol. 2012 Dec 26; 365(1-2):64.

Oxygen and Placental Cells

Both preeclampsia and low birthweight are caused in part by placentas that develop poorly as a result of insufficient blood flow from the mother to the placenta. This low blood flow causes low levels of oxygen at the placental surface. While low oxygen is normal in the first trimester, insufficient oxygen in the second trimester can cause various changes in the placenta and lead it to express proteins that can affect the mother's blood pressure. To study what happens when there is insufficient oxygen, trophoblast cells (the placental cells directly in contact with mother's blood) were cultured under three different oxygen levels, <,1%, 8% and 20%. We found that a small subset of genes showed an increase of DNA methylation when cultured in hypoxic (<1% oxygen) conditions. Many of the specific sites that changed were places where another protein, AP-1, binds to the DNA. We also found that the components of this protein showed increased expression in the cells cultured under low oxygen. We suggest that AP-1 causes DNA methylation to accumulate at the sties that it binds to, shutting down the associated genes and causing the cells to change their growth direction. Understanding the early steps in the process leading to abnormal placentation may lead to novel therapies to reduce their occurrence.

Yuen RK, Chen B, Blair JD, Robinson WP, Nelson DM. Hypoxia alters the epigenetic profile in cultured human placental trophoblasts. Epigenetics, 2013 Jan 11;8(2).

Newsletter Summer 2013

Robinson Lab *Reproductive Genetics Research at UBC and the Child & Family Research Institute*

Neural Tube Defects (NTDs)

Through our study of epigenetic marks in the placenta, we are trying to understand how the placenta adapts to poor nutrition to help keep the baby healthy. We are using new techniques to study these epigenetic changes at the vast majority of known genes, and have developed new approaches to learn more about the biological processes that underlie changes in epigenetic marks in more detail.

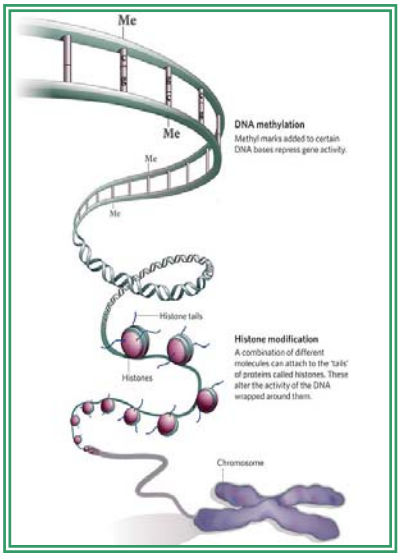
Adapted from: php.med.unsw.edu.au

We are currently applying this knowledge to the study of neural tube defects (NTDs), a birth defect influenced by both genetic and environmental factors. NTDs occur when the neural tube does not close completely and are associated with reduced folate intake in the mother. Folate is an important nutrient for cell growth and division in early embryonic development and for generating the normal epigenetic changes during development. However, NTDs are still occurring despite extensive folate supplementation. Do some cases of NTDs occur independently of the folate metabolism pathway? Or is the available maternal folate simply not getting to the growing embryo where it is needed? We are hoping to learn about folate metabolism and DNA modification in placentas of pregnancies affected with NTDs in order to understand why they occur and to develop new prevention strategies. We have found that there is a reduction in DNA methylation in the placentas of pregnancies affected by NTDs. There are also changes in DNA methylation at a subset of specific genes. To look at these changes it is helpful to compare DNA methylation at many different sites. This work is being done by Dr. Maria Penaherrera and PhD student Magda Price who are using the 450K epigenome analysis described next (in collaboration with Dr. D. McFadden, Dr. A. Devlin and Dr. M. Van Allen). We will use this information to identify what biological changes occur to cause NTDs and to determine if there is a subset of NTD cases that are folate-independent in etiology.


Nutritionduringpregnancy.wordpress.com

450K Epigenome Analysis

In the last 10 years researchers have been able to conduct large-scale genetic studies that show changes in DNA sequence that can be linked to disease. A complimentary area of research is large-scale "epigenome" studies that can show us chemical changes to DNA that do not alter the sequence itself. Studying these chemical changes has become very important in medical research since they may be involved in turning genes on and off and may also be sensitive to environmental factors (like age, exposure to chemicals, and nutrition). DNA methylation is one such chemical change that researchers believe may be a way in which the environment can affect our DNA. Arrays are tools that can be used to take a snapshot of this chemical modification at hundreds of thousands of sites throughout the genome. The array we use in our studies can look at more than 485,000 sites. However, some of these sites may not measure what was intended when the array was designed. Magda Price, PhD student, and several collaborators, worked to identify those sites that were compromised and ease the interpretation of data obtained using a DNA methylation array. We found that between 8 and 13% of the array sites may not measure what was intended, and therefore should not be included in this analysis. We also added information to all sites that will help researchers understand the meaning of the data they get from the array. The analysis of large arrays is very complicated and the scientific community is debating the best steps for studying this type of data. As more researchers begin to use this array tool, it becomes increasingly important that issues related to quality and interpretation of array data are discussed so that valuable research is produced.



embryology.med.unsw.edu.au

Price EM, Cotton AM, Lam LL, Farreé P, Robinson WP, Kobor MS. Additional annotation enhances potential for biologically-relevant analysis of the Illumina Infinium HumanMethylation450 BeadChip array. Epigenetics Chromatin. 2013 Mar 3; 6(1):4.

Newsletter Summer 2013

Robinson Lab *Reproductive Genetics Research at UBC and the Child & Family Research Institute*

Recurrent Miscarriage and Imprinting

Miscarriage, the spontaneous loss of pregnancy before 20 weeks, is the most common complication of pregnancy. Chromosome imbalances (abnormal number or structure of chromosomes) in the developing placenta or fetus account for 50% or more of miscarriages; however, the cause of the chromosomally normal miscarriages is poorly understood. As appropriate DNA methylation is essential for development of a healthy baby, we hypothesized that abnormal DNA methylation may cause chromosomally normal miscarriage, particularly among women experiencing 3 or more consecutive losses. Courtney Hanna, a PhD in our lab, specifically investigated DNA methylation patterns in the placenta, due to its critical role in the health of pregnancy. When comparing DNA methylation between placentas from 10 miscarriages and 10 controls, we identified differences at several genes, including some involved in immune response and fetal/placental growth. We followed this up in a larger set of 70 miscarriage samples and observed unusual overall patterns of DNA methylation in 10% of samples, including both miscarriages and controls. Further studies are needed to understand if such changes can contribute to poor placental function in miscarriage.

www.hhc.rca.ac.uk

Hanna CW, McFadden DE, Robinson WP. DNA methylation profiling of placental villi from karyotypically normal miscarriage and recurrent miscarriage. Am J Pathol. 2013 Jun; 182(6):2276-84.

Recurrent Miscarriage, Triploidy and Gene Mutation

There are many causes of pregnancy loss that involve chromosomal abnormalities, two of which include molar pregnancies and triploidy. A **molar pregnancy** is a form of pregnancy complication in which a fertilized egg ends up with an abnormal number of chromosomes and is non-viable. In a healthy pregnancy both the mother and the father will donate 23 chromosomes to the fertilized egg. Molar pregnancies can happen in two ways: an egg without chromosomes from the mother can be fertilized by a sperm and receive only chromosomes from the father (a complete molar pregnancy), or the egg can have a normal number of chromosomes from the mother, and receive a double set from the father (a partial molar pregnancy). In either case a fetus will not develop normally, and instead a mass develops in the uterus. **Triploidy** is another disorder resulting from an abnormal number of chromosomes. In the case of triploidy an embryo will have three copies of every chromosome instead of the normal two. Most babies affected by triploidy do not live to term. The causes of these pregnancy complications are largely unknown, but Irina Manokhina, a post-doc in our lab has been studying the suggested association with two genes, *NLRP7* and *C6orf221*.

Gene Mutations and Miscarriage

Irina Manokhina (post doc) recently published a paper looking at mutations in two genes *NLRP7* and *C6orf221. T*hese genes are known as "maternal –effect genes", which are genes that are produced in the egg and are needed for the early development of the embryo. Mutations in *NLRP7* and *C6orf221* have been found in most women who experience a specific type of molar pregnancy called "biparental hydatidiform moles". It has been suggested that mutations to these genes may also play a role in other adverse pregnancy outcomes, including other types of moles, polyploidy (complete extra sets of chomosomes), recurrent spontaneous abortions and stillbirths of uncertain etiology. Irina's study examined these associations in hopes of better understanding the causes of molar pregnancies, triploidy and recurrent miscarriage, and to investigate the potential for screening for mutations to *NLRP7* and *C6orf221* in women with adverse reproductive outcomes. Results did not show these two genes to be major contributing factors to these causes of miscarriage, and more studies are needed to determine if screening women for these mutations would be an effective part of reproductive care.

Manokhina I, Hanna CW, Stephenson MD, McFadden DE, Robinson WP. Maternal NLRP7 and C6orf221 variants are not a common risk factor for androgenetic moles, triploidy and recurrent miscarriage. Mol Hum Rep, 2013 Apr 4; 0(0):1-6.

Newsletter Summer 2013

Robinson Lab *Reproductive Genetics Research at UBC and the Child & Family Research Institute*

SELECTED PUBLICATION LIST (LAST 3 YEARS)

1. Price EM, Cotton AM, Lam LL, Farré P, Emberly E, Brown CJ, Robinson WP, Kobor, MS. Additional annotation enhances potential for biologically relevant analysis of Illumina Infinium Human Methylation 450 BeadChip array. Epigenetics Chromatin. 2013 Mar 3; 6(1):4.
2. Hogg K, Blair JD, von Dadelszen P, Robinson WP. Hypomethylation of the LEP gene in placenta and elevated maternal leptin concentration in early onset pre-eclampsia. Mol Cell Endocrinol. 2013 Mar 10;367(1-2):64-73.
3. Yuen RK, Chen B, Blair JD, Robinson WP, Nelson DM. Hypoxia alters the epigenetic profile in cultured human placental trophoblasts.Epigenetics. 2013 Jan 11;8(2).
4. Hogg K, Price EM, Hanna CW, Robinson WP. Prenatal and perinatal environmental influences on the human fetal and placental epigenome. Clin Pharmacol Ther. 2012 Dec;92(6):716-26.
5. Price EM, Cotton AM, Peñaherrera MS, McFadden DE, Kobor MS, Robinson W. Different measures of "genome-wide" DNA methylation exhibit unique properties in placental and somatic tissues. Epigenetics. 2012 Jun 1;7(6):652-63.
6. Peñaherrera MS, Jiang R, Avila L, Yuen RK, Brown CJ, Robinson WP. Patterns of placental development evaluated by X chromosome inactivation profiling provide a basis to evaluate the origin of epigenetic variation. Hum Reprod. 2012 Mar 19
7. Hanna CW, Bloom MS, Robinson WP, Kim D, Parsons PJ, Vom Saal FS, Taylor JA, Steuerwald AJ, Fujimoto VY. DNA methylation changes in whole blood is associated with exposure to the environmental contaminants, mercury, lead, cadmium and bisphenol A, in women undergoing ovarian stimulation for IVF. Hum Reprod. 2012 Feb 29
8. Hanna CW, Blair JD, Stephenson MD, Robinson WP. Absence of SYCP3 mutations in women with recurrent miscarriage with at least one trisomic miscarriage. Reprod Biomed Online. 2012 Feb;24(2):251-3
9. Yuen RK, Manokhina I, Robinson WP. Are we ready for DNA methylation-based prenatal testing? Epigenomics. 2011 Aug;3(4):387-90.
10. Novakovic B, Yuen RK, Gordon L, Penaherrera MS, Sharkey A, Moffett A, Craig JM, Robinson WP, Saffery R. Evidence for widespread changes in promoter methylation profile in human placenta in response to increasing gestational age and environmental/stochastic factors. BMC Genomics. 2011 Oct 28;12:529.
11. Yuen RK, Jiang R, Peñaherrera MS, McFadden DE, Robinson WP. Genome-wide mapping of imprinted differentially methylated regions by DNA methylation profiling of human placentas from triploidies. Epigenetics Chromatin. 2011 Jul 13;4(1):10.
12. Cotton AM, Lam L, Affleck JG, Wilson IM, Peñaherrera MS, McFadden DE, Kobor MS, Lam WL, Robinson WP, Brown CJ. Chromosome-wide DNA methylation analysis predicts human tissue-specific X inactivation. Hum Genet. 2011 Aug;130(2):187-201. Epub 2011 May 20.
13. Yuen RK, Neumann SM, Fok AK, Peñaherrera MS, McFadden DE, Robinson WP, Kobor MS. Extensive epigenetic reprogramming in human somatic tissues between fetus and adult. Epigenetics Chromatin. 2011 May 5;4:7.
14. Yuen RK, Robinson WP. Review: A high capacity of the human placenta for genetic and epigenetic variation: implications for assessing pregnancy outcome. Placenta. 2011 Mar;32 Suppl 2:S136-41. Epub 2011 Feb 1. Review.
15. Avila L, Yuen RK, Diego-Alvarez D, Peñaherrera MS, Jiang R, Robinson WP. Evaluating DNA methylation and gene expression variability in the human term placenta. Placenta. 2010 Dec;31(12):1070-7. Epub 2010 Oct 14
16. Yuen RK, Peñaherrera MS, von Dadelszen P, McFadden DE, Robinson WP. DNA methylation profiling of human placentas reveals promoter hypomethylation of multiple genes in early-onset preeclampsia. Eur J Hum Genet. 2010 Sep;18(9):1006-12. [Epub ahead of print May 5.]
17. Hanna CW, Bretherick KL, Liu CC, Stephenson MD, Robinson WP. Genetic variation within the hypothalamus-pituitary-ovarian axis in women with recurrent miscarriage. Hum Reprod. 2010 Oct;25(10):2664-71.
18. Peñaherrera MS, Weindler S, Van Allen MI, Yong SL, Metzger DL, McGillivray B, Boerkoel C, Langlois S, Robinson WP. Methylation profiling in individuals with Russell-Silver syndrome. Am J Med Genet A. 2010 Feb;152A(2):347-55.
19. Bourque DK, Avila L, Peñaherrera M, von Dadelszen P, Robinson WP. Decreased placental methylation at the H19/IGF2 imprinting control region is associated with normotensive intrauterine growth restriction but not preeclampsia. Placenta. 2010 Mar;31(3):197-202. Epub 2010 Jan 8.
20. Robinson WP, Peñaherrera MS, Jiang R, Avila L, Sloan J, McFadden DE, Langlois S, von Dadelszen P. Assessing the role of placental trisomy in preeclampsia and intrauterine growth restriction. Prenat Diagn. 2010 Jan;30(1):1-8.

Newsletter Summer 2013

Robinson Lab *Reproductive Genetics Research at UBC and the Child & Family Research Institute*

HOW TO PARTICIPATE IN OUR RESEARCH

www.babiesandtoddlers.com.au

We are currently recruiting for a variety of studies:

- **CONTROL WOMEN WITH UNCOMPLICATED PREGNANCIES**
- **CONTROL WOMEN WITH SUCCESSFUL PREGNANCIES AT/OVER AGE 37**
- **ABNORMAL MATERNAL SERUM SCREEN, PREECLAMPSIA and/or IUGR PREGNANCY**
- **WOMEN WITH RECURRENT MISCARRIAGES OR A TRISOMIC PREGNANCY**
- **WOMEN WITH PREMATURE OVARIAN FAILURE (POF)**
- **PRENATAL DIAGNOSIS OF NEURAL TUBE DEFECT**
- **PRENATAL or POSTNATAL DIAGNOSIS OF TRISOMY MOSAICISM**
- **IMPRINTING DISORDERS AND UNIPARENTAL DISOMY**
- **DIAGNOSIS OF HYDATIDIFORM MOLE, TRIPLOIDY or PLACENTAL MESENCHYMAL DYSPLASIA**

To find out more about our research studies please contact **us at:**

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